愛知教育大学

AICHI UNIVERSITY OF EDUCATION

Effects of Gibberellic Acid on Growth and Development, and the Genetic Study in Common Bean (*Phaseolus vulgaris* L.), Applicable for Cambodian High School

A thesis

In Partial Fulfilment of the Requirement for the degree of

Master of Biological Education

219M060

Phen Sarith

Mach, 2021

愛知教育大学

AICHI UNIVERSITY OF EDUCATION

Effects of Gibberellic Acid on Growth and Development, and the Genetic Study in Common Bean (*Phaseolus vulgaris* L.), Applicable for Cambodian High School

A thesis

In Partial Fulfilment of the Requirement for the degree of

Master of Biological Education Science

Supervisor: Professor Dr. Juntaro KATO

Examination Committee:

……

……

219M060

Phen Sarith

Mach, 2021

Table of Content

CHAPTER 4

LINKAGE ANALYSIS OF INCOMPLETE DOMINANT PAIR, FLOWER AND STEM COLOR IN COMMON BEAN (*PHASEOLUS VULGARIS* L.) BY DIRECT RECOMBINATION VALUE ESTIMATION

CHAPTER 5

LINKAGE ANALYSIS OF INCOMPLETE DOMINANCE AND DOMINANCE CHARACTER PAIR, FLOWER COLOR AND STEM LENGTH, STEM LENGTH AND STEM COLOR, AND STEM COLOR AND FLOWER COLOR IN COMMON BEAN (*PHASEOLUS VULGARIS* L.) BY MAXIMUM LIKELIHOOD ESTIMATION METHOD

LIST OF FIGURE

List of Tables

Abbreviation

GAs	gibberellin acid (GA_1, GA_3, GA_4, GA_7, and GA_9).
HAI	the code of F_1 plants in the experiment, referred from the name of Haibushi cultivar.
UNESCO	United Nations Educational, Scientific and Cultural Organization.
STEM	Science, Technology, Engineering and Mathematic.
SL	Science Literacy.
TEL	Technology and Engineering Literacy.
ML	Mathematic Literacy.
ST Gibera	the name of Japanese commercial gibberellic acids (GA_3), used in the experiment.
B-NINE	the name of Japanese commercial gibberellic inhibitor used in the experiment.
DW	distilled water, used as the control treatment.
A' and B'	referred the models of the experiments in gibberellin inhibitor B-NINE.
F_1	the Mendel's hybridization (first generation progeny).
F_2	the Mendel's second generation progeny.
st^{pig}-fl^{pig}	the alleles pair of gene pigment referred to the plant with purple and red in stem and flower color of male parent characteristics.
g-w	the alleles pair of the gene pigment referred to the plants with green and white in stem and flower color of female parent characteristic.
MLE	the maximum likelihood estimation method used in characteristic analysis.
MoEYS	the Ministry of Education, Youth and Sport
PBL	Project-Based Learning approach.

Abstract

In Cambodia high school, plant hormone and genetics study only are learned by textbook without experiments. It may occur little understanding for students in these chapters. In this study, to produce experimental class, I used common beans, which have many cultivars with different traits, to perform exogenous GAs and GAs inhibitor treatment and cross for genetics study. In General introduction described the study of the effects of Gibberellic Acid (GAs) on plant growth which is studied in Cambidian12th grade biology, and the study of Mendel's genetics and the gene linkage. The review of GAs and its function and the genetic theory was also described in this chapter. Even though the effects of GAs on plant growth and genetics were studied, there were less experimental classes and research activity in these fields in high school in Cambodia. My study aims to promote biology experiments and researches activities in Cambodian high school education.

In the physiological chapter, GAs effect was confirmed by exogenous GA treatment and GA inhibitor treatment. Common bean vine-less cultivar 'Morocco' was used to research the effects of GA. The experiments were designed in two models A, plant was sprayed the GA solution once a week, and model B, plant was sprayed GA solution twice a week. The 1mg/L, 5mg/L, and 10mg/L GA solutions were used in these two experimental models. Common bean vine cultivar 'Haibushi' was used to research the effects of plant growth inhibitor "B-NINE." The experiments were also designed in two models, A' and B'. In model A', each plant was sprayed with 0.08g/L, 0.4g/L, or 0.8g/L B-NINE solutions once a week. In model B', each plant was sprayed 0.16g/L, 0.8g/L, or 1.6g/L B-NINE concentration twice a week. The result showed that GA promoted stem and internode elongation of vine-less cultivar Morocco depended on GA concentrations in both model A and model B experiments, and there were significant differences between model A and model B. The effects of GA to promote reproductive organs and other vegetative organs development were not clear through these experiments. The result of experiments about the effects of B-NINE on vine cultivar Haibushi in both models A' and model B' showed good results that B-NINE had potent inhibition on stem and internodes elongation decrease depended on concentration with significant differences. The effects of B-NINE on reproductive organs developed were also not clear statistically, but B-NINE seemed to promote the induction of flower number.

In genetics chapter included Mendelian laws and linkage analysis. In this study, the vine cultivar 'Haibushi', which had purple stem and red flower color, crossed with the vines-less cultivar 'Morocco' which have a green short stem and white flower color. The crossing techniques were developed for applications in a high school biology experiment. The result showed that F_1 had normal stem, mixed color and pink flower color. F_2 populations obtained from all F_1 plants has the ratio, 1:2:1 of purple: mixed: green stem plants in stem color and also 1:2:1 of red: pink: white

flower plants in flower color (Mendel's 2nd law). In stem length characteristics, the F_2 population obtained from two F_1 plants (12-HAI and 15-HAI) were segregated in the ratio 3:1 of normal stem and short stem plants (Mendel's 2nd law). However, the F_2 population obtained from 14-HAI, 11-HAI, and 8-HAI did not segregate in the ratio 3:1. So, these F_2 populations were assumed by two vine-less (short) gene loci. Genotypes of the parents were estimated to $d_1d_1d_2d_2$ or $d_1d_1D_2d_1$ tentatively in this study. When these three F_1 plants had the genotype $D_1d_1D_2d_2$ (normal stem plant), the F_2 populations could segregate with the ratio: 9:7 =normal stem: short stem plants (Mendel's 3rd law). In the results of chi-square tests indicated that there were no significant differences between observed values and expected values. In Cambodian high school textbook, there are no description of genetic epistasis. So, not only Mendel's 3rd law but also genetic epistasis can be taught to use in this cross combinations. In the same cross combination, stem color and flower color were also different from these parents. The vine cultivar Haibushi with purple stem and red flower was crossed with the vine-less cultivar Morocco with green stem and white flower. In the F_2 populations, because all combinations between stem color and flower color were not followed Mendel's 3rd law of independence, thus presence of genetics linkage were considered. Because these two characters showed incomplete dominance, all combination of genotypes could be estimated. When recombinant value was designated as *r*, then *r*-value was estimated by direct estimation methods, 'recombinant estimated chromosome'/'total estimated chromosomes'. The formula to calculate the recombination rate (*r*) to calculate the expected F_2 genotype population to calculate the chi-square test was introduced in this linkage analysis. Chi-square test of four of five populations did not detect significant differences between observed value and expected value, between two gene, stem color and flower color, linked and map distance calculation was 9.7%-21.6%. Although it is impossible to estimate genotypes from phenotypes in two gene combination of F_2 population with complete dominance, estimations of genotypes from phenotype might be possible in two gene combination of F_2 population with incomplete dominance. The combination of stem color and flower color in common bean might be able to use for experimental study of linkage genetics in high school.

Chapter 1

General Introduction

1.1 The educational framework development

Many developed countries have already highly developed their unit education system and then global STEM standards proposed. The UNESCO competency framework, STEM literacy, was adapted as the global education policy for 21st century development (UNESCO, 2011). The STEM literacy's domain included science literacy (SL), technology engineering literacy (TEL), and mathematic literacy (ML). From science literacy, biology improves STEM education and STEM life standards (AES 2018). Cambodian high school biology education adapted to the ideologies from those whom scientific proposed. However, the teaching method seems more provided the textbook reading in the classroom than experiment and field activities to integrate students' understanding. The lacking scientific achievement due to curriculum content reforming is not yet correct and teaching methodologies. Thus these two criteria teaching method and curriculum content development are crucial for improving Cambodian high school education.

1.2 Cambodian biology textbook in high school

In the Cambodian biology textbook, gibberellic acid (GAs) has been adopted as the study content in the 12th grade in high school education. The textbook was described in chapter 4 as the protein's function in the organ and included the amino acid and enzyme (Yahoo *et al*., 2016). The chapter described the form and function; however, the experiment activities were not primarily applied. On the other hand, the genetic study chapter adapted in the 11th grade of the high school textbook. The chapter described Mendelian ideology (Mendel., 1866) and Thomas H. Morgan (1911). Mendel's proportion 3:1 explained as 2nd law and 9:3:3:1 as the 3rd law, but genetic epistasis, which showed the proportion of 9:7, 9:3:4, 9:6:1, was not explained yet in Cambodian high school biology textbook. Due to yet of the GA_3 apply the experimentation and Mendel’s crossing; thus, this study on the effects of gibberellic acid (GAs) and GAs inhibitor on growth and development and the genetic study on common bean cultivar are significant Cambodian biology in high school education.

1.3 Gibberellic acid and function

The gibberellin acid (GA) is one of five types of plant and fungal hormone, while those four are being auxins, cytokinin, ethylene, and abscisic acid. The role function of GAs is primarily studied in the plant parts, while its role in fungal, however, is not yet clearly understood. Any research strongly supports two parts, "seed germination" and "stem elongation," while other roles being debate. The role in germination, GAs, is functioned to break down of starch to glucose in the

endosperm begin short time after the seed is exposed to water, while the role stem elongation cause higher levels of transcription of the gene coding for the amylase enzyme to stimulate the synthesis of amylase. GAs are an essential tool used for researchers worldwide to establish the specific function. More than 130 GAs forms in plants, fungi, and bacteria to date; only a subset, namely GA_1, GA_3, GA_4, GA_7, and GA_9, were thought to function as bioactive hormones. Additional forms of GAs in plants were precursors of the bioactive forms or deactivated metabolites (Binenbaum *et al*., 2018). Most of the GAs in plants, focuses on plant development were stem length elongation, fruit, flower, and seed germinate but less with the study of plant inhibitory parts. Thus this research hypothesizes that the gibberellin inhibitor B-NINE effects on vegetative and reproductive organs of common bean vine cultivar Haibushi (Futaba Syubyo, Japan). This research hypothesizes that the gibberellin inhibitor B-NINE affects vegetative and reproductive organs of the common bean vine cultivar Haibushi. This study is trying to find out the difference of stem elongation, the number of branches, leave, leaves size, and the number of internode of common bean vine cultivars Haibushi, which there was no reporting yet.

1.4 Genetic theory review

Gregor J. Mendel was the first describe the plant genetic study in 1866. His scholarly research was basically on pea plant crossing. He found that the inheritance of the offspring characteristic came from the parent's characteristics. He established three laws of genetic inheritance, the law of dominance, law of segregation, and law of independent assortment. He also found the intermediate characteristic of the offspring, which represents incomplete dominance characteristics. The other geneticist, Thomas Hunt Morgan, established the genetic linkage in 1911 used a fruit fly backcross. He conducted the backcross of F_1 with the homo recessive parent. In the F_2 generation, the proportion of segregation not followed Mendel's 3^{rd} law, 9:3:3:1. Thus he hypothesized that linkage between parent's genes would present. Finally, Thomas H. Morgan published the theoretical linkage in 1911.

1.5 The aim of the study

This study aims to promote biology contents and apply experiment for biology science in Cambodian high school biology education. The experimentations proposed to find out the effects of gibberellic acid, GA_3, and gibberellin inhibitor B-NINE on growth and development, and the genetic study in common bean, which applicable for Cambodian high school education.

Chapter 2

Effects of Gibberellic Acid (GA_3) and Gibberellin Inhibitor (B-NINE) on Growth and Development in Common Bean Cultivars (*Phaseolus vulgaris* L.)

2.1 Introduction

Gibberellic acid (GA) was specific plant hormones that positively regulate plant growth. The function of gibberellic acid describes as the plant hormones in regulation growth known as early as the 1950s (Brian and Hemming., 1955; Chudasama and Thaker., 2007). Moreover, the study of GAs developed by many researchers scholar the majority, function, and form. The function of GAs forms determined in developing stem elongation (Yamaguchi *et al*., 1998; and Leite *et al*., 2003), seed germination (Leite *et al*., 2003; and Khafagi *et al*., 2018), other parts growth of the plants (Chudasama and Thaker., 2007) and expansion through cell growth, trichome development, the transition from vegetative to reproductive growth, flower, seed, and fruit development (Weinstein and Shani., 2018). The gibberellins are commercially used to enhance phenotypic characteristics, earliness, and productivity of many vegetable and ornamental crops (Miceli *et al*., 2019). Thus in this study, the gibberellic acid and gibberellin inhibitor were used to confirm the effects on plant characteristics of the vine and vine-less common bean for producing experiment class in Cambodian high school education.

2.2 Materials and methodology

2.2.1 Plant materials

Two cultivars of common bean, cv. Haibushi, which had vine and cv. vine-less Morocco were used. The vine cultivar, Haibushi, was used to change for vine-less characteristics by applying GA inhibitor B-NINE, while the vine-less cultivar Morocco used to change the character for vine by endogenous GA apply.

2.2.2 Chemical materials

In this study, ST Gibera Tablets 5 (Sumitomo Chemical Horticulture, Japan) used as gibberellic acid for restore of dwarf phenotype, and B-NINE (Daminozide; Nisso Green Co., Ltd.) used to produce artificial dwarf phenotype (vineless) on common bean.

2.2.3 Experimental equipment

The measurement tools used in this experiment were a digital electric scale for seed weighting (Figure 2.1d), a classic fibber tape meter of 10 meters in length for stem length measurement (Figure 2.1e), a digital calliper model LifeLex Stainless Hardened with 150mm of potential

maximum length and ±0.003mm of accuracy for internode measurement (Figure 2.1f) and a presser plastic bottle for spraying the plant hormone on the experimental plants (Figure 2.1g).

Figure 2.1 The experiment equipment and materials. a) plants materials, b) B-NINE, c) ST Gibera GA_3, d) Scale, e) tape meter, and f) digital calliper, and g) Presser plastic bottle.

2.2.4 Chemical dilution

ST Gibera Tablets 5 (GA_3) dilution

First, take one tablet of ST Gibera Tablets 5 (GA_3) to dilute in distilled water (DW) to produce the 50ml solution stock-1. Then use the injection syringe, pump 0.5ml, 2.5ml, and 5ml from solution

stock-1 to dilute in DW produce 50ml of the final solution in different concentrates 1mg/L, 5mg/L, and 10mg/L as bellow:

- Pump 0.5ml of solution stock-1 diluted with DW to produce 50ml of final concentrate (1mg/L GA_3).
- Pump 2.5ml of solution stock-1 diluted with DW to produce 50ml of final concentrate (5mg/L GA_3).
- Pump 5ml of solution stock-1 diluted with DW to produce 50ml of final concentrate (10mg/L GA_3).

B-NINE Dilution

Method 1 (used for experiment model A'): take one powder package (1g) of B-NINE to dissolve in DW to produce the 100ml solution stock-1. Then use the injection syringe, pump 0.5ml, 2.5ml, and 5ml from solution stock-1 to produce three different B-NINE concentrates as bellow:

- Pump 0.5ml of solution stock-1 dissolved with DW to produce 50ml of final concentrate (0.08g/L B-NINE).
- Pump 2.5ml of solution stock-1 dissolved with DW to produce 50ml of final concentrate (0.4g/ B-NINE).
- Pump 5ml of solution stock-1 dissolved with DW to produce 50ml of final concentrate (0.8g/L B-NINE).

Method 2 (used for experiment model B'): take one powder package (1g) of B-NINE to dissolve in DW to produce the 50ml solution stock-1. Then use the injection syringe, pump 0.5ml, 2.5ml, and 5ml from solution stock-1 to produce three different B-NINE concentrates as bellow:

- Pump 0.5ml of solution stock-1 dissolved with DW to produce 50ml of final concentrate (0.16g/L B-NINE).
- Pump 2.5ml of solution stock-1 dissolved with DW to produce 50ml of final concentrate (0.8g/L B-NINE).
- Pump 5ml of solution stock-1 dissolved with DW to produce 50ml of final concentrate (1.16g/L B-NINE).

2.2.5 Chemical treatments

GA_3 treatments

These exogenous GA_3 treatments experimentations were designed into two models, A and B.
Model A to spray GA_3 solution or distilled water once a week (one week interval).
Model B to spray GA_3 solution one time every two weeks (two weeks interval).

There were four kinds of concentration including control in each model by spraying 3ml of distilled water, 1 mg/L, 5 mg/L, or 10 mg/L of GA_3. There were five plants (n=5) used in each treatments, thus total twenty plants for model A, and other twenty plants for model B.

B-NINE treatments

The treatment of plant growth inhibitor, B-NINE, was also designed into two models, A' and model B'.

Model A' to spray B-NINE solution of 3ml for one plant for once a week.
Model B' to spray the same volume of B-NINE as in model A', for twice a week.

There were four treatments including control, 0.08g/L B-NINE, 0.4g/L B-NINE, or 0.8g/L B-NINE in model A’ and half concentration of B-NINE were used in model B’. There were 5 plants in each treatment so the total plants for each model were twenty plants.

2.2.6 Measuring method

Internode measuring the young plants' internodes used in this experiment measured before the experimental start (week-0) using the digital calliper, model LIFE-LEX STAINLESS HARDENED, with 150mm maximum length. The internode was measured by starting from the middle of a node to the middle of the next node. The internodes were measured weekly after the experimental start.

Leave size measuring leaf size was measured when the plants had two true-leaves two weeks after seedlings stage. The leaf size was measured by focusing on the longest width and its most extended length using the digital calliper. The leaf size was measured every week.

Flower counting the number of flowers were visual counted only once at two weeks after transplanting to the field (flowering season).

Seed weighting and seed size measuring the seed was weighted using the digital scale, and seed size was measured using a digital calliper by the width and length of seed.

2.2.7 Statistical analysis

Significant differences among each treatments were analysed using free software, Real Statistic Using Excel (Charles Zaiontz), in version of one-factor ANOVA at HSD follow up option with p-value<0.05.

2.3 Result and discussion

2.3.1 The of effects GA_3

2.3.1.1 The vegetative organs growth Figure 2.2 showed the plant growth at week-4 after GA_3 treatment in 3 different concentrations and control. From these figures, they are easy to observe that GA_3 of different concentrations had different effects on stem length elongation. The higher concentration of GA_3 promotes higher stem length elongation. However, statistical data analysis showed that the length of the internodes, the length of the stem, leaf size, leaf number, and the number of branches were studied in these experiments.

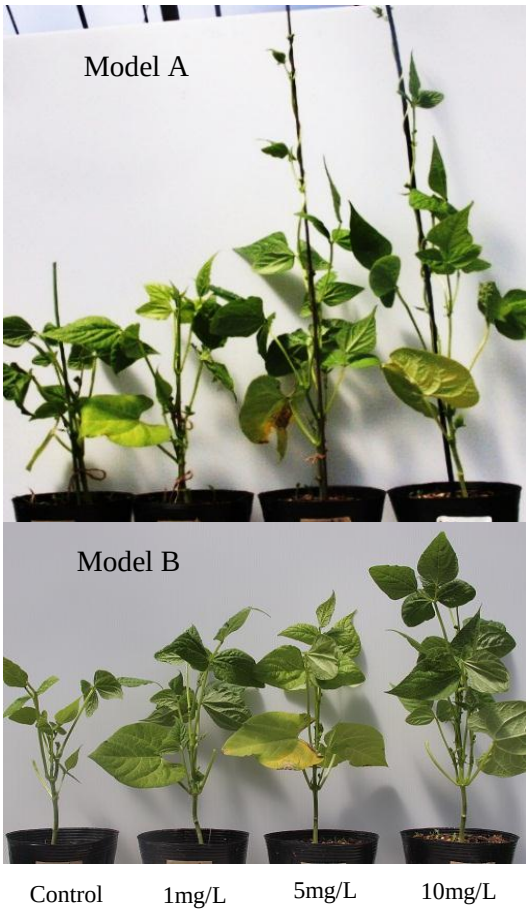


Figure 2.2 The plants height of model A and model B after week-4 of different GA_3 concentrate treatments.

Stem elongation

Model A Figure 2.3 showed that plant height from week-1 to week-2 were similar between control and all those three GA_3 treatments 1mg/L, 5mg/L, and 10mg/L. However, from week-3 to week-4, the result showed that control and 1mg/L were still similar in plant height, while other two treatments 5mg/L and 10mg/L GA_3, were taller than the plants that received distilled water and 1mg/L GA_3. In week-4, the effects of GA_3 on stem length elongation showed that plants received GA_3 treatments in any concentrations. The plant received 10mg/L GA_3 was longest and following by plants that received 5ml/L and 1mg/L GA_3, respectively (Figure 2.3). Furthermore, there were significant differences between plants received 5mg/L, and plants received 10mg/L GA_3 compared to control plants by statistical analysis, p-value<0.05. These results showed that a high concentration of GA_3 treatment promoted stem length elongation of plants.

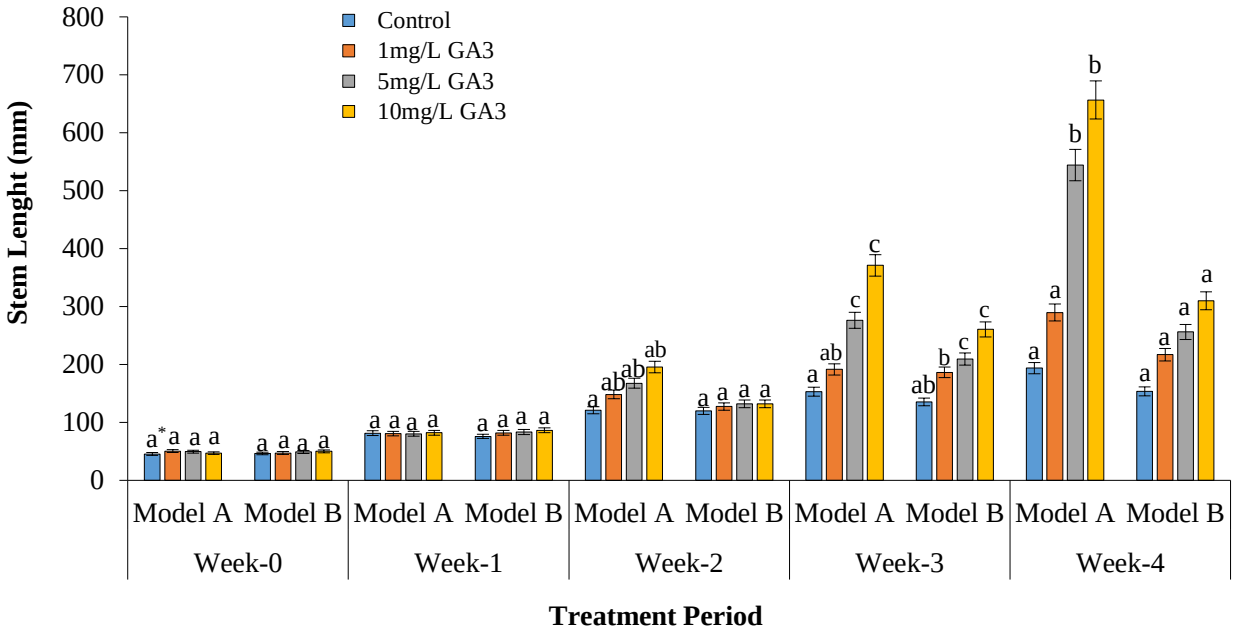


Figure 2.3 Effects of GA_3 on stem length elongation.
* Different letters on bars charts showed the significant differences among the treatments in model and across the model at the same week, by one-factor ANOVA at HSD follow up option at p-value <0.05.

Model B The stem length elongation were promoted by GA_3 treatments in week-3 and week-4. The plants received a higher concentration of GA_3 elongated longer orderly. Plants that received GA_3 and control were significantly differences between control and 1mg/L GA_3 with 5mg/L and 10mg/L GA_3 by the statistical analysis at week-3, p-value<0.05 (Figure 2.3).

Model A and model B experiments indicated that a high concentration of GA_3 from 5mg/L and higher had significant effects on stem length elongation. Since model A, each plant received a double amount of GA_3 concentrates compared to model B's, plants in model A elongated longer than model B's. From these two model experiments, the effects of GAs on stem length elongation studied in biology textbook in Cambodia were confirmed. The effects of GA_3 on stem length elongation were caused by meristem by increasing cell number more than cell enlarges (Mitsuro., 1964). Stem length elongation was caused by increasing GA_3 biosynthesis that reacted as the catalysis to promote cell division (Mitsuro., 1964). Tanimoto *et al* (2006) reported that GA_3 affected stem length elongation of pea plants, and Jaques *et al* (2019) also reported that GA_3 affected bean growth. Recently, Mam *et al* (2019) reported that GA_3 had significant effects on dwarf tomato stem length elongation. Lester *et al* (1997) reported that gene *Le* caused stem length elongation. The report showed that gene *Le* of pea plant encoded by gibberellin *3ß-Hydroxylase* (GA_4) of *Arabidopsis*.

Internode length and internodes number

The number of internodes in each plant was counted at week-4 in the experiment. Result showed that the average number of the internodes in all treatments of model A and model B were numerical

hierarchy from lowest (controlling) to the highest (10mg/L) statistically. The average number of model A's internodes was increasing orderly from control=6.80, 1mg/L=7.40, 5mg/L=8.80, to 10mg/L=9.60. The average number of internodes of model B were control=6.20, 1mg/L=7.40, 5m/L=8.00, to 10mg/L=8.60 (Figure 2.4).

In these experiments, internodes 1 and 2 have already existed before GA_3 treatment. Figure 2.4 and table 2.1 showed that the length of internodes 1 and 2 in all treatments, including control, were not different. This indicated that GA_3 did not have any effects on old internode elongation. In model A, internodes 3 of week-2, internodes of plants received GA_3 in all concentrations elongated a little longer than control plant's internode. Moreover, the effects of GA_3 on internode elongation were observed from internodes 4 to 7, and the plants that received higher concentration GA_3 elongated in longest. In model B, the effects of GA_3 on internode elongation were observed from internodes 4, and also plants received higher concentration of GA_3 its internodes elongated longer orderly (Figure 2.4 and Table 2.1). The effects of GA_3 on internode elongation found in this experiment were similar to the effects of GA_3 on internode elongation in tomato plants reported by Mam *et al* (2019).

Since the number of internodes was not significantly differences between plants that received GA_3 and plants in control indicated that stem length elongation of plants that received GA_3 was caused by internodes elongation, but not by increasing internode numbers.

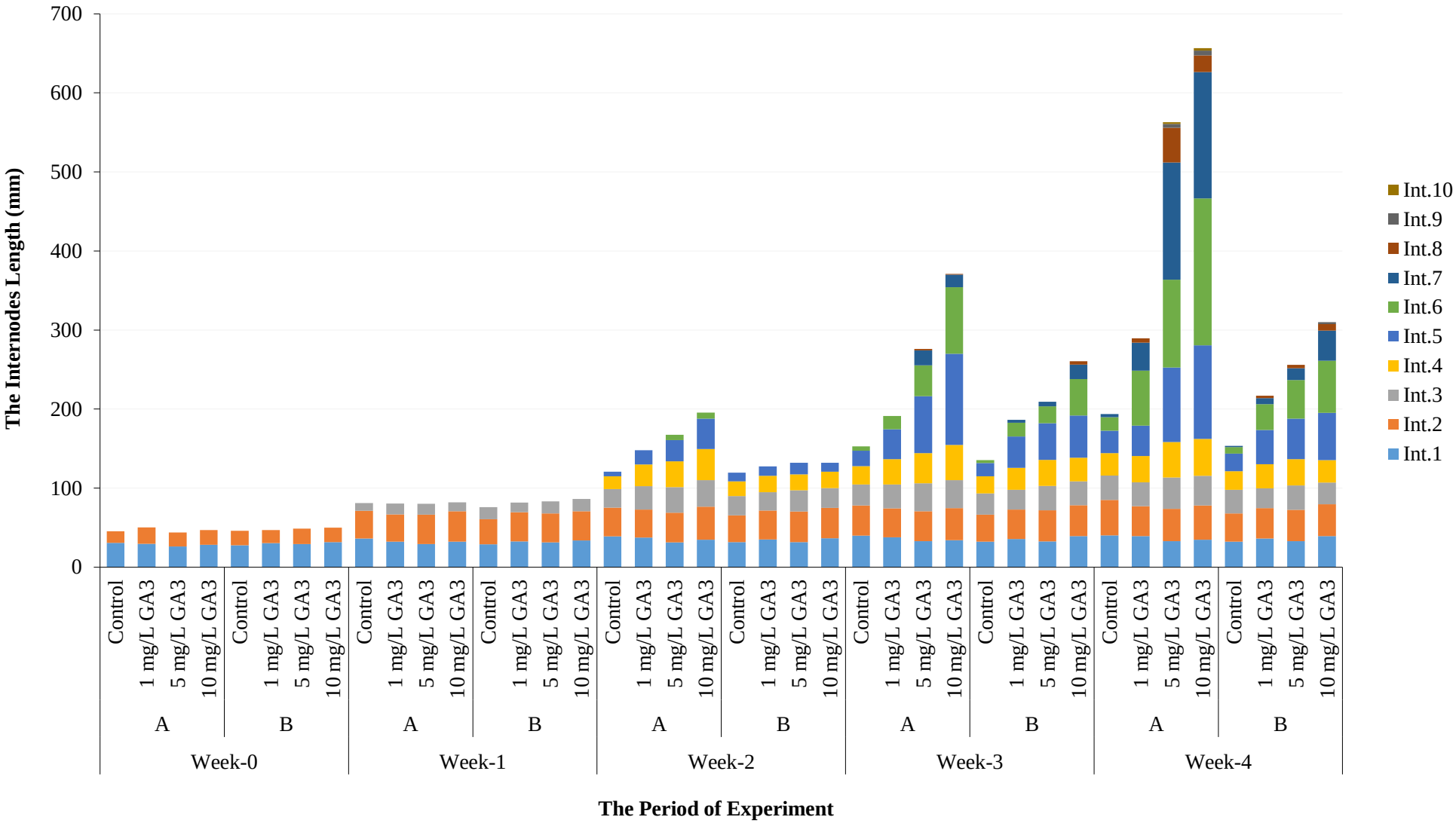


Figure 2.4 The effect of GA_3 on internodes length and internodes number**.**

Table 2.1 Average and standard deviation of internode length of vine-less common bean Morocco cv. at different weeks after GA_3 treatment (mm ± SD).

Measurement Period	Internodes	Model A								Model B							
		Control		1mg/L GA_3		5mg/L GA_3		10mg/L GA_3		Control		1mg/L GA_3		5mg/L GA_3		10mg/L GA_3	
		Ave.	***SD***	***Ave.***	***SD***	***Ave.***	***SD***	***Ave.***	***SD***	***Ave.***	***SD***	***Ave.***	***SD***	***Ave.***	***SD***	***Ave.***	***SD***
Week-0	Int.1	30.71	± 3.22	29.66	±8.61	26.06	±3.54	28.42	±5.69	27.86	±9.20	30.54	±6.50	29.40	±3.95	31.74	±2.04
	Int.2	14.71	±1.82	20.67	±2.42	17.82	±4.76	18.55	±2.01	18.26	±4.92	16.44	±3.11	19.47	±4.79	18.39	±1.58
Week-1	Int.1	36.38	±4.52	32.36	±8.97	29.10	±3.08	32.21	±5.58	28.97	±8.67	32.61	±6.83	31.32	±4.79	33.87	±2.49
	Int.2	35.03	±5.63	34.35	±3.44	37.42	±3.02	38.50	±4.17	31.59	±9.40	37.00	±1.63	36.66	±3.00	36.95	±6.99
	Int.3	9.84	±1.49	13.87	±8.09	13.70	±2.92	11.25	±5.95	15.26	±5.05	12.21	±1.05	15.31	±0.91	15.44	±5.09
Week-2	Int.1	38.91	±2.99	37.52	±7.71	31.35	±2.81	34.68	±5.21	31.56	±9.46	34.91	±7.91	31.62	±4.88	36.55	±2.43
	Int.2	36.29	±6.09	35.36	±3.57	37.59	±3.62	41.98	±5.45	33.89	±11.31	36.77	±2.48	38.82	±4.13	38.54	±5.06
	Int.3	23.52	±3.30	29.46	±7.04	32.37	±8.70	33.51	±2.90	24.58	±6.77	23.14	±2.30	26.98	±7.35	25.10	±1.72
	Int.4	16.18	±5.04	27.70	±9.40	32.63	±11.97	39.17	±10.93	18.41	±8.32	20.83	±5.77	20.16	±6.13	20.65	±2.90
	Int.5	5.83	±4.82	17.99	±12.20	26.73	±20.66	38.57	±15.10	11.22	±6.86	11.75	±7.61	14.47	±14.86	11.31	±2.33
	Int.6	0	0	0	0	6.83	±5.62	7.63	±3.03	0	0	0	0	0	0	0	0
Week-3	Int.1	40.03	±3.91	37.84	±8.72	33.06	±3.86	34.23	±5.52	32.23	±10.08	35.68	±8.52	32.74	±5.69	39.17	±2.94
	Int.2	38.00	±6.74	36.68	±1.49	37.74	±3.60	40.40	±3.98	34.14	±11.32	37.18	±2.98	39.29	±3.79	39.25	±5.15
	Int.3	26.59	±2.28	30.18	±6.92	35.25	±7.81	35.35	±2.32	26.99	±5.98	24.98	±2.92	30.89	±3.37	30.02	±7.82
	Int.4	23.27	±3.07	31.96	±8.69	38.34	±9.29	44.75	±10.73	21.55	±8.94	27.84	±6.22	32.67	±2.28	29.90	±3.34
	Int.5	19.41	±5.98	37.75	±17.88	71.80	±35.43	115.17	±22.20	16.98	±10.70	39.80	±15.81	46.40	±8.47	53.59	±10.98
	Int.6	5.66	±3.00	17.01	±19.67	39.19	±28.73	84.39	±31.30	3.40	±3.30	17.24	±10.30	21.47	±3.73	45.89	±10.05
	Int.7	0	0	0	0	18.76	±18.30	15.76	±6.62	0	0	3.53	±3.23	5.81	±0.87	18.45	±6.73
	Int.8	0	0	0	0	1.82	±3.64	0.98	±1.96	0	0	0	0	0	0.00	4.25	±2.60
Week-4	Int.1	40.12	±3.83	39.33	±8.51	32.79	±2.42	34.69	±5.45	32.27	±10.09	36.18	±8.82	32.96	±5.54	39.22	±2.95
	Int.2	44.93	±7.05	37.72	±2.91	40.87	±4.92	43.30	±4.72	35.62	±11.54	38.61	±2.70	39.58	±3.75	40.51	±3.23
	Int.3	30.79	±4.76	30.26	±6.91	39.82	±3.55	37.62	±3.61	30.11	±5.43	25.08	±3.37	30.95	±6.29	27.40	±2.44
	Int.4	28.48	±4.59	33.34	±9.19	44.76	±7.15	46.64	±11.59	23.41	±7.53	30.40	±7.71	33.15	±1.41	28.42	±14.12
	Int.5	28.33	±8.33	38.50	±17.90	94.20	±17.66	118.46	±22.78	22.39	±12.26	43.34	±17.74	51.29	±6.42	59.60	±6.86
	Int.6	16.94	±8.25	69.47	±42.45	111.09	±58.15	185.90	±38.83	8.15	±6.19	32.48	±19.24	48.72	±7.83	66.12	±9.93
	Int.7	4.03	±2.22	35.52	±47.19	148.42	±77.98	159.77	±44.04	1.48	±2.03	7.79	±4.86	14.96	±6.52	38.05	±12.84

Int.8	0	0	5.50	±5.15	43.99	±32.13	21.12	±4.04	0	0	2.99	±1.71	4.34	±0.58	8.29	±3.23
Int.9	0	0	0	0	4.75	±2.85	6.14	±3.76	0	0	0	0	0	0	2.35	±2.15
Int.10	0	0	0	0	2.14	±1.97	2.79	±2.43	0	0	0	0	0	0	0	0

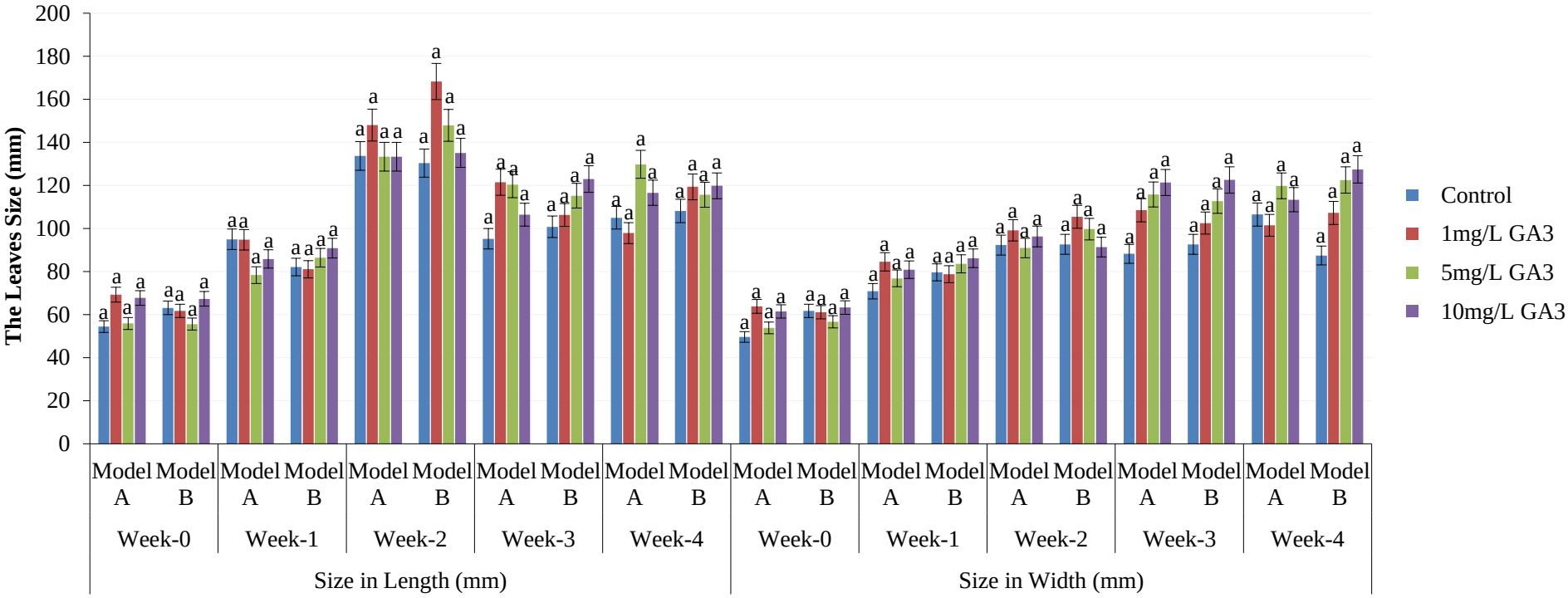


Figure 2.5 The average of leaves size**.**

Leaves size and leaves number

Figure 2.5 showed that GA_3 of any concentrations did not affect leaf size. However, the results showed that the plants that received GA_3 treatments have slightly longer and broader than the control plants. The effects of GA_3 on leaf size by different GA_3 were unclear because sometimes the plants that received lower concentration of GA_3 had more giant leaves than the plants received higher concentration of GA_3 (Figure 2.5). On the other hand, statistical tests among all GA_3 treatments and the control plants were no significant differences. However, Ngatia *et al* (2004) reported that GA_3 positively affects leave area index (LAI). Therefore, further research on GA_3 effects on the size of leaves of common bean should be conducted.

The number of leaves of plants that received GA_3 was generally increased more than the control plants in both Model A and model B from week-3 (Figure 2.6). However, there were no significant differences by statistical tests at p-value<0.05. However, Ngatia *et al* (2004) and Noor *et al* (2017) studied the effects of GAs on leave growth. Their result showed that GA_3 increased the left area index (LAI) and the number of leaves. Therefore, the experiment by spraying higher concentration of GA_3 on common should be conducted more in order to see the significant effects of GA_3 on plants to induce leaf number.

Number of branches

The number of branches was counted in the week-4 of the experiment. Data from both models A and model B showed that the number of branches increased when the plants received a higher concentration of GA_3 treatment. The number of model A branches was control=3, 1mg/L=3, 5mg/L=5, and 10mg/L=6 branches (Figure 2.6); while the model B was control=2, 1mg/L=4, 5mg/L=5, and 10mg/L=5 branches (Figure 2.6). There were significant differences between plants that received 10mg/L compared to plants that received distilled water and plants that received 1mg/L GA_3 (p-value<0.05). This result showed the effects of GA_3 increase number of branches when a high concentration of GA_3 was treated on the plants. Ferdowsi *et al* (2017) studied the effects of GA_3 on *P. Vulgaris* L. Their result showed that the GA_3 at 30-90ppm (around 5-10mg/L) promoted the increasing number of branches. In this experiment, the higher concentration of GA_3 has stronger effects on increasing the number of branches. From this study and the study conducted by Ferdowsi *et al* (2017), a high concentration of GA_3 have significant effects on branches inducing.

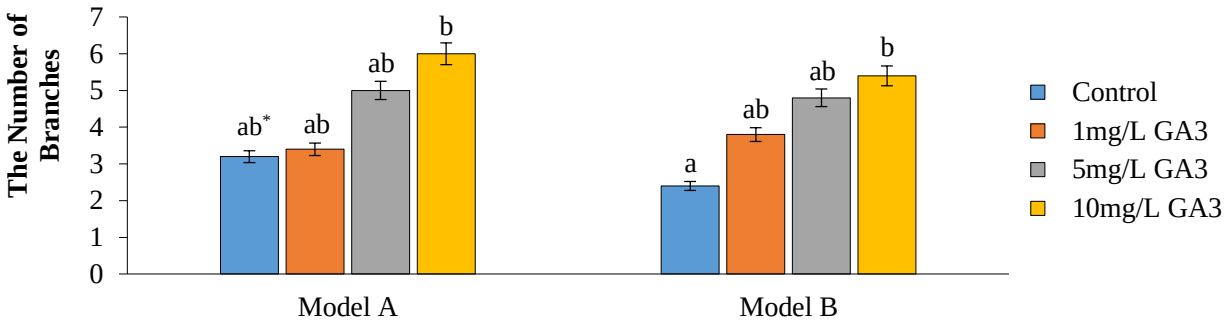


Figure 2.6 Effects of GA_3 on number of branches**.** * Different letters on the bars showed the significant differences among the treatments in the model and in the across model at the same week, by one-factor ANOVA at HSD follow up option at p-value <0.05.

2.3.1.2 Reproductive organs development

Number of flowers

This data was recorded only one time duringg flower season. GA_3 treatment did not have significant effects on common bean to induce flowers in model A. The average number of flower buds from model A were control=17.60, and GA3 treatments 1mg/L=12.20, 5mg/L=20.60, and 10mg/L=18.20 (Figure 2.7a). In model B, the plants received higher concentration of GA_3 treatment induced higher amount of flowers. The number of flowers in the model B were control=9.20, 1mg/L=9.60, 5mg/L=16.20, and 10mg/L=25.40. There was significant difference of flower number between the plants received 10ml/L GA_3 and the plants received lower concentration of GA_3 and distilled water (Figure 2.7a).

Seed size

Size in length the result showed that the plants received GA_3 treatments of all concentrations had similar seed length compared to the seeds of the control plants in both models A and model B. In these experiments in both model A and model B indicated that the seeds of plant received GA_3 were shorter than the seeds of the plants that did not receive GA_3 (Figure 2.7b). However, there were not significant differences statistically analysed with p-value <0.05.

Size in width GA_3 treatments on the common bean did not show the different seed width and their effects were not clear. Even though there were some differences in seed width among the treatments, there were not significant differences through statistical analyses (Figure 2.7b).

Seeds weight

The results of the experiments in both models A and model B showed that lower concentration of GA_3 treatments promoted seed weight comparing to seeds produced by plants received higher concentration of GA_3 or distilled water. However, there were not significant differences in

statistical analysis (Figure 2.7c). However, the quality and the amount of seeds were not sufficient enough because there was strong raining within the period of plants producing and the fruit collection. Thus further experiments about the effects of GA_3 on common bean cv. vine-less Morocco to produce seeds should be conducted.

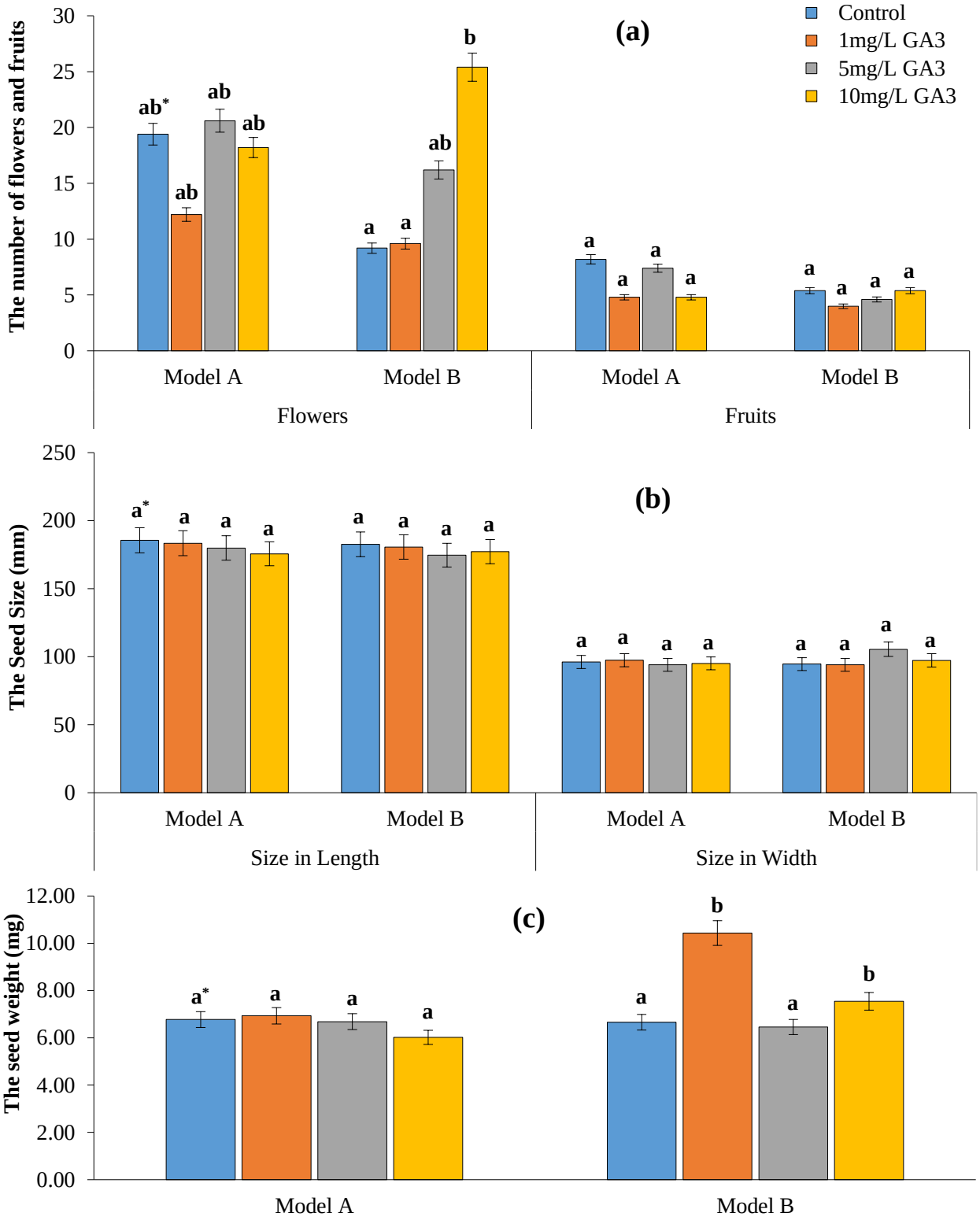


Figure 2.7 The GA3 effected on reproductive organs development. a) the number of flower, b) the seed size, and c) the seed weight. * Different on the bar charts showed the significant different among the treatments in model and across models experiments at the same week, by one-factor ANOVA at HSD follow up option at p-value<0.05.

2.3.2 Effects gibberellin inhibitor B-NINE

2.3.2.1 Vegetative organs growth

Figure 2.8, model A' showed the plants received lower concentration of B-NINE with spraying one time a week, the plants received higher concentration of B-NINE inhibited stem elongation in comparison to the control and lower concentration of B-NINE. Figure 2.8, model B' which higher concentration (twice) compared to model A', and the plants were sprayed two time a week. The plants received higher concentration from 0.8g/L and 1.16g/L B-NINE were inhibited the stem elongation more compared to the plants received lower concentration of B-NINE and the control plant.

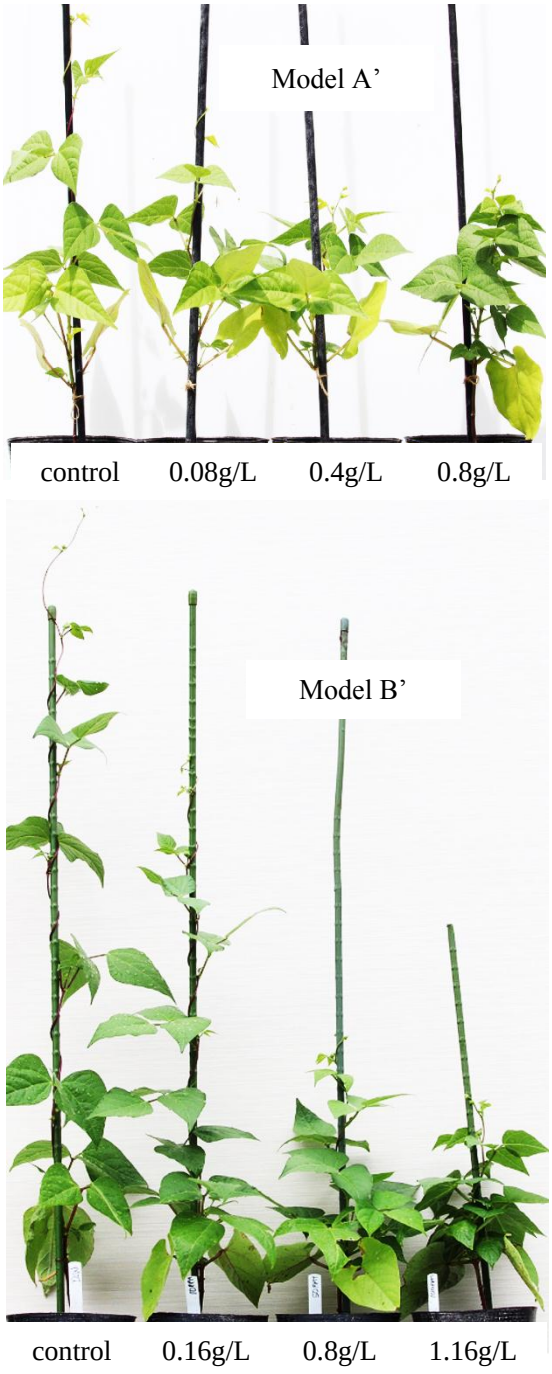


Figure 2.8 Plants height at week-4 of B-NINE exogenous spraying.

Stem length elongation

Experiment model A', All plants received B-NINE were inhibited stem elongation compared to the control plants from week-2 after spraying B-NINE and the stronger inhibition was observed from week-3 to week-4 (Figure 2.8, model A' and table 2.2 model A'). In week-4, the plants received higher concentration of B-NINE (0.8g/L) grew shorter than the plants received lower concentration of B-NINE (0.4g/L). The expected result, the plants received higher concentration of B-NINE should be shorter than the plants received higher concentration. However, there was no significant differences among all treatments in this experimental model (Figure 2.9, model A').

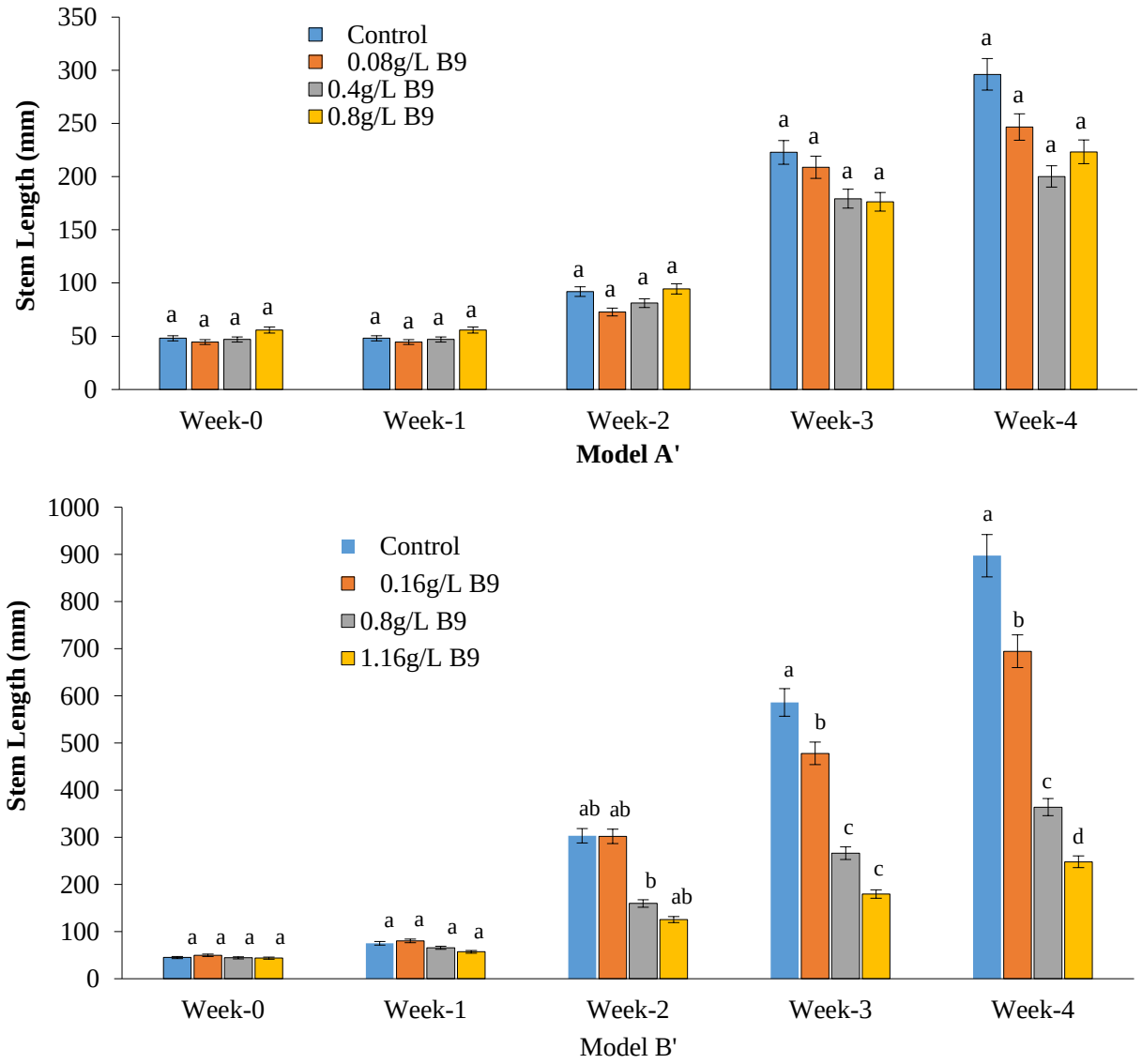


Figure 2.9 Stem length elongation**.**

Experiment model B', The inhibition effects of B-NINE on stem elongation were observed in the 1st week (week-1) after the plants received B-NINE treatment, and the significant differences between the plants received 0.8g/L or 1.16g/L B-NINE and the control plants were observed from week-2 (Figure 2.9, model B' and Table 2.2, model B'). The effects of B-NINE on stem elongation were very different among the plants received different concentration of B-NINE and also between the control plants. From these two experimental Models, the result showed that the plants that received higher concentration of B-NINE were inhibited the stem elongation more than those received lower concentration. Model B' is a good experimental methods to be applied for biological experimental class in high school, because the significant effects of B-NINE were very different from one treatment to another treatment which can prevent students from confusion. Jabir *et al* (2017) reported that B-NINE has the more effective inhibition of the vegetative aerials part of plant growth than the underground parts of the plant. Rademacher (1991) reported that B-NINE

functioned by primary blocking to the plant's biosynthetic pathways. Liu and Hou (2018) reported that the plant growth inhibitor, B-NINE, reduced vegetative organ growth.

Number of internodes

The results of both models A' and model B' showed that B-NINE did not effect on number of internodes inducing. In model A', the average number of internodes produced by the plants in different treatments were the same (10 internodes) (Figure 2.10, model A'). In model B', the number of internodes also were not different between the control plants and the plants received B-NINE treatment (Figure 2.10, model B'). From these two model experiments showed B-NINE did not have any effects on number of internodes producing.

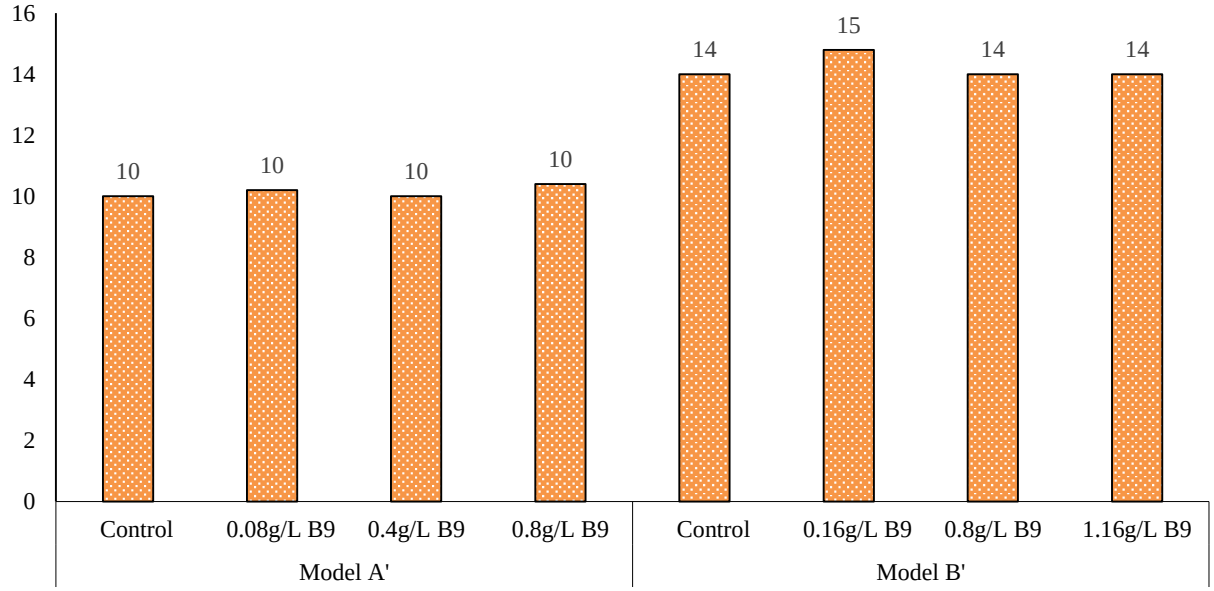


Figure 2.10 Number of internodes.

Length of internodes

In model A', the effects of inhibitor B-NINE on internode elongation were observed from internodes number 5, number 6 and number 7. The internodes of the plants received B-NINE were shorter than the internodes of the control plants at week-3 and week-4 (Figure 2.11, model A').

In model B', the effects of B-NINE to inhibit the internode elongation were observed from internodes number 2 from the one week (week-1) after the plants received B-NINE treatment (Figure 2.11, model B'). The plants received higher concentration of B-NINE were inhibited the stem elongation more than the plants received lower concentration of B-NINE.

This experiment showed that the inhibition of stem elongation was caused by the inhibition of internodes because the number of internodes of all treatments in this experiment were not different from each other (Figure 2.11).

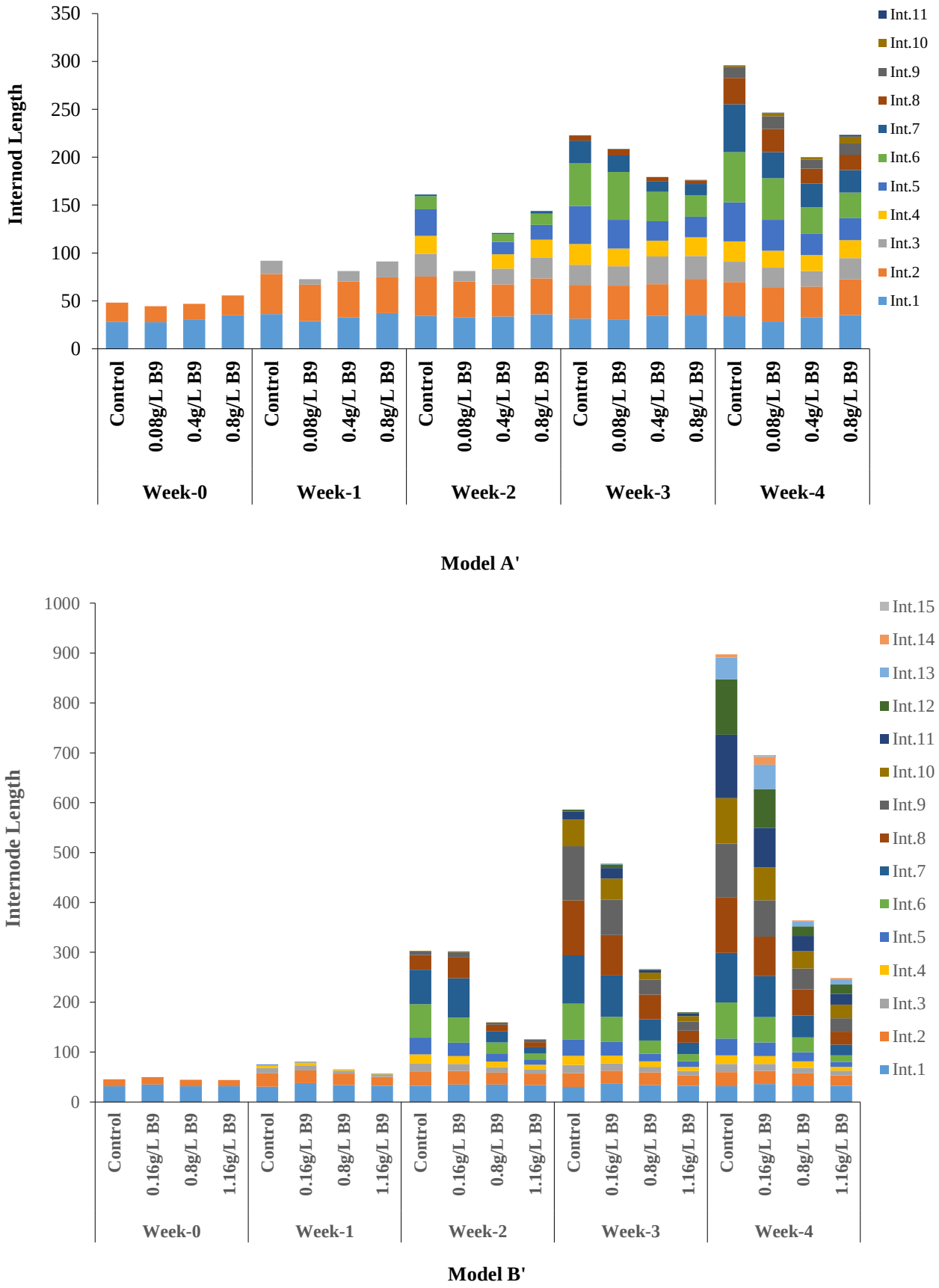


Figure 2.11 Effects of B-NINE (B9 in figure) inhibitor on internodes elongation.

Table 2.2 Average and standard deviation of internode length of common bean cultivar Haibuhi from different weeks of gibberellic acid inhibitor B-NINE treatments (mm ± SD).

Measurement Period	Number of Internodes	Model A'								Model B'							
		Control		0.08g/L B_9		0.4g/L B_9		0.8g/L B_9		Control		0.16g/L B_9		0.8g/L B_9		1.16g/L B_9	
		Ave	***SD***	***Ave***	***SD***	***Ave***	***SD***	***Ave***	***SD***	***Ave***	***SD***	***Ave***	***SD***	***Ave***	***SD***	***Ave***	***SD***
Week-0	Int.1	27.98	±6.13	27.69	±10.89	30.27	±7.11	34.84	±14.04	31.55	±2.73	34.90	±3.91	31.41	±2.52	31.77	±3.05
	Int.2	20.17	±17.67	16.80	±14.93	16.73	±15.95	20.89	±11.28	13.70	±3.01	15.12	±4.40	13.18	±1.47	12.30	±4.53
Week-1	Int.1	36.21	±4.48	28.90	±3.42	32.95	±7.27	37.17	±10.61	30.71	±3.55	37.96	±3.71	33.88	±2.48	32.71	±3.73
	Int.2	41.76	±7.27	38.10	±8.16	37.33	±9.14	37.55	±7.43	26.91	±1.66	25.45	±1.14	22.96	±2.35	17.37	±6.52
	Int.3	13.98	±5.78	5.70	±8.34	10.81	±3.87	16.40	±5.38	10.37	±0.80	9.69	±1.40	5.87	±1.26	5.41	±1.58
	Int.4	0	0	0	0	0	0	3.30	±6.60	5.96	±0.70	6.01	±1.12	2.87	±1.54	1.82	±1.54
	Int.5	0	0	0	0	0	0	0	0	1.38	±1.82	1.33	±1.62	0	0	0	0
Week-2	Int.1	34.12	±3.87	32.95	±7.27	33.78	±7.56	35.97	±7.56	32.54	±2.81	35.06	±4.70	34.80	±1.24	34.09	±5.20
	Int.2	41.35	±3.81	37.33	±9.14	33.29	±5.14	37.57	±6.19	28.66	±1.78	27.42	±2.65	24.22	±2.48	22.66	±3.95
	Int.3	23.56	±6.20	10.81	±3.87	16.46	±3.60	21.86	±2.15	16.19	±1.01	13.46	±0.85	10.95	±1.45	9.03	±1.68
	Int.4	18.94	±5.84	0	0	15.14	±8.01	18.57	±4.93	18.13	±1.84	16.07	±2.21	10.75	±1.50	9.01	±1.51
	Int.5	27.54	±13.01	0	0	12.90	±6.00	15.37	±5.89	33.48	±8.36	26.94	±3.41	16.50	±3.96	10.29	±0.68
	Int.6	13.81	±11.96	0	0	8.11	±5.21	11.69	±5.32	66.96	±26.79	50.10	±11.46	22.27	±6.83	11.58	±0.95
	Int.7	1.67	±3.34	0	0	1.12	±2.24	2.82	±3.53	69.09	±54.61	78.94	±18.07	22.06	±8.25	13.19	±2.64
	Int.8	0	0	0	0	0	0	0	0	29.45	±30.35	41.53	±17.68	13.38	±4.73	10.03	±3.00
	Int.9	0	0	0	0	0	0	0	0	7.34	±4.96	11.30	±3.31	4.31	±2.94	4.62	±3.08
	Int.10	0	0	0	0	0	0	0	0	1.39	±1.75	1.36	±1.81	0.55	±1.09	1.28	±1.56
Week-3	Int.1	31.39	±3.93	30.29	±7.22	34.16	±5.68	35.41	±8.56	30.27	±2.45	36.52	±3.54	34.12	±2.02	32.48	±3.31
	Int.2	35.30	±6.54	35.62	±7.20	33.34	±5.56	37.54	±6.18	27.77	±1.70	26.19	±1.96	25.11	±2.19	21.30	±3.90
	Int.3	21.02	±3.88	20.34	±5.05	28.85	±26.49	24.03	±4.75	16.28	±1.36	14.31	±1.25	11.08	±1.20	8.71	±1.91
	Int.4	21.61	±5.78	18.36	±5.83	16.63	±8.08	19.32	±4.33	18.24	±2.47	15.85	±1.85	11.00	±1.32	7.93	±1.75
	Int.5	39.89	±14.58	29.97	±15.28	20.19	±8.97	21.63	±4.35	33.15	±7.50	27.23	±3.47	15.51	±4.12	11.11	±0.89
	Int.6	44.42	±22.13	49.88	±29.24	30.68	±23.49	22.24	±7.69	71.18	±23.38	50.60	±11.25	26.30	±5.80	14.40	±0.85
	Int.7	23.68	±14.45	17.44	±14.59	11.11	±5.91	11.97	±4.77	97.00	±34.73	83.17	±14.89	42.85	±11.65	22.71	±2.63
	Int.8	5.50	±3.82	6.43	±5.69	4.31	±2.16	3.65	±3.03	110.43	±20.17	80.76	±5.99	49.40	±18.40	24.08	±4.46
	Int.9	0	0	0.56	±1.12	0	0	0.55	±1.10	108.34	±17.17	70.56	±12.50	29.74	±15.22	18.22	±3.45

	Int.10	0	0	0	0	0	0	0	0	53.23	±13.6	42.61	±23.37	14.18	±5.28	11.26	±3.49
	Int.11	0	0	0	0	0	0	0	0	16.50	±7.28	21.36	±9.94	5.58	±1.02	5.44	±1.30
	Int.12	0	0	0	0	0	0	0	0	3.94	±3.06	7.06	±3.83	1.38	±1.13	2.03	±1.14
	Int.13	0	0	0	0	0	0	0	0	0	0	2.02	±1.74	0	0	0	0
Week-4	Int.1	33.85	±3.62	28.4	±7.52	32.92	±6.22	35.11	±8.03	31.5	±2.00	35.94	±3.83	33.13	±1.42	32.43	±3.00
	Int.2	35.82	±6.69	35.33	±7.33	32.07	±7.50	37.62	±6.56	28.21	±1.56	26.23	±1.26	24.26	±2.62	20.9	±4.11
	Int.3	21.13	±4.19	20.93	±3.91	16.14	±3.32	21.95	±2.26	16.24	±1.20	14.11	±1.35	11.1	±1.47	9.22	±2.21
	Int.4	21.46	±5.62	17.68	±5.60	16.69	±7.61	18.76	±4.44	17.61	±2.18	15.74	±2.04	12.93	±5.07	7.82	±1.02
	Int.5	40.55	±14.47	32.35	±16.41	22.61	±8.88	22.99	±3.86	33.61	±8.21	27.45	±3.35	18.42	±6.45	9.93	±0.85
	Int.6	52.42	±16.79	43.35	±28.16	27.33	±13.75	26.61	±6.80	72.13	±22.05	50.55	±11.20	29.68	±11.27	12.91	±1.29
	Int.7	50.16	±27.80	27.28	±17.63	24.79	±14.07	23.52	±6.20	99.29	±34.95	82.03	±13.67	43.72	±11.53	21.87	±2.81
	Int.8	27.3	±27.39	24.26	±22.85	15.61	±8.84	16.02	±2.16	110.9	±21.00	79.46	±5.84	52.41	±16.39	25.87	±3.45
	Int.9	11.77	±16.70	13.31	±17.42	9.26	±5.81	11.72	±3.77	108.6	±15.23	72.99	±9.62	41.78	±13.06	26.61	±3.39
	Int.10	1.55	±3.11	3.07	±4.65	2.71	±2.79	7.16	±3.89	91.26	±10.97	66.19	±14.29	34.86	±4.63	27.52	±1.06
	Int.11	0	0	0.62	±1.24	0	0	1.758	±2.15	127.1	±23.88	78.92	±13.02	30.75	±4.97	21.73	±9.66
	Int.12	0	0	0	0	0	0	0	0	110.9	±36.66	77.81	±18.51	19.13	±6.60	18.69	±2.53
	Int.13	0	0	0	0	0	0	0	0	43.93	±31.77	48.77	±24.16	9.45	±5.70	8.91	±1.18
	Int.14	0	0	0	0	0	0	0	0	6.33	±3.23	14.92	±10.23	2.57	±2.14	3.77	±0.93
	Int.15	0	0	0	0	0	0	0	0	0	0	3.51	±2.70	0	0	0	0

Number of branches

The number of branches from model A’ was observed slightly different on week-3 and week-4 through control and all B-NINE treatments, while model B's were similar. However, the number of branches from model B was more than model A's. Data analysis showed no difference between control with B-NINE treatments 0.16g/L, 0.8g/L, and 1.6g/L at p-value> 0.05 from both model A and model B (Figure 2.12). In this result study on the effectiveness of inhibition B-NINE seems like well development on branches increasing. There were significant differences across B-NINE treatments from model A’, while model B’ was not. However, both model A’ and model B’ indicated that this inhibition B-NINE affected branches' growth respectively. Few studies confirm this functional inhibition of B-NINE on branch increasing.

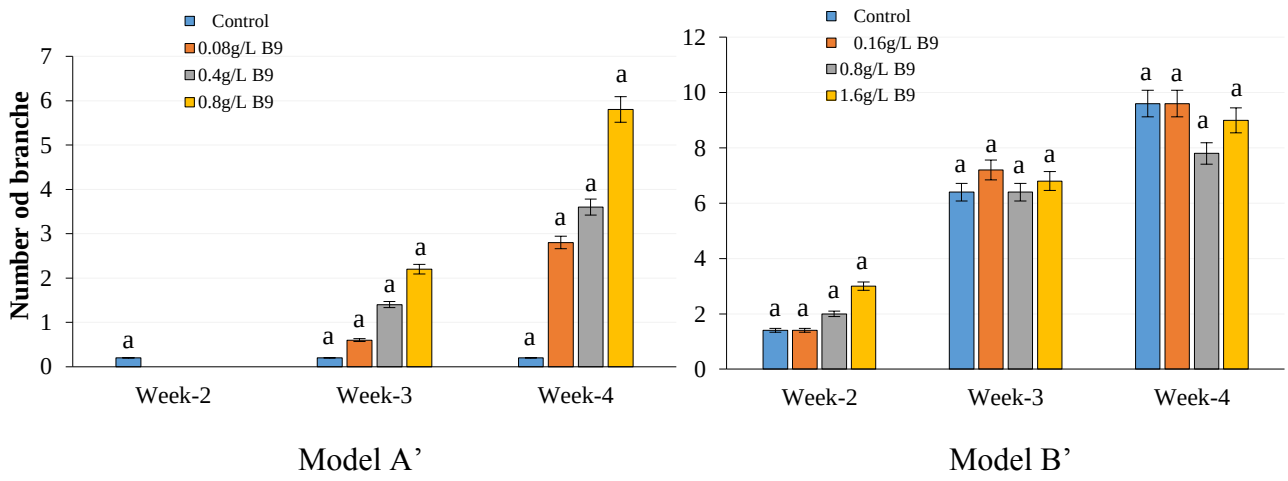


Figure 2.12 Effects of B-NINE (B9 in figure) inhibitor on number of branches inducing.

Number of leaves

The growth inhibitor, B-NINE did not inhibit the number of leaves. In model A’, the plants received B-NINE were slightly increased the number of leaves compared to the control plants at week-3 and week-4 (Figure 2.13, model A’). In model B’, the plants treated by B-NINE slightly increased the number of leaves compared to the control plant at week-3, but they were slightly inhibited at week-4 (Figure 2.13, model B’). However, there were not significant differences by statistical test among the treatments in both models A’ and B’. Based on the result from these experiments, B-NINE did not have any significant effects on the plants to produce the number of leaves.

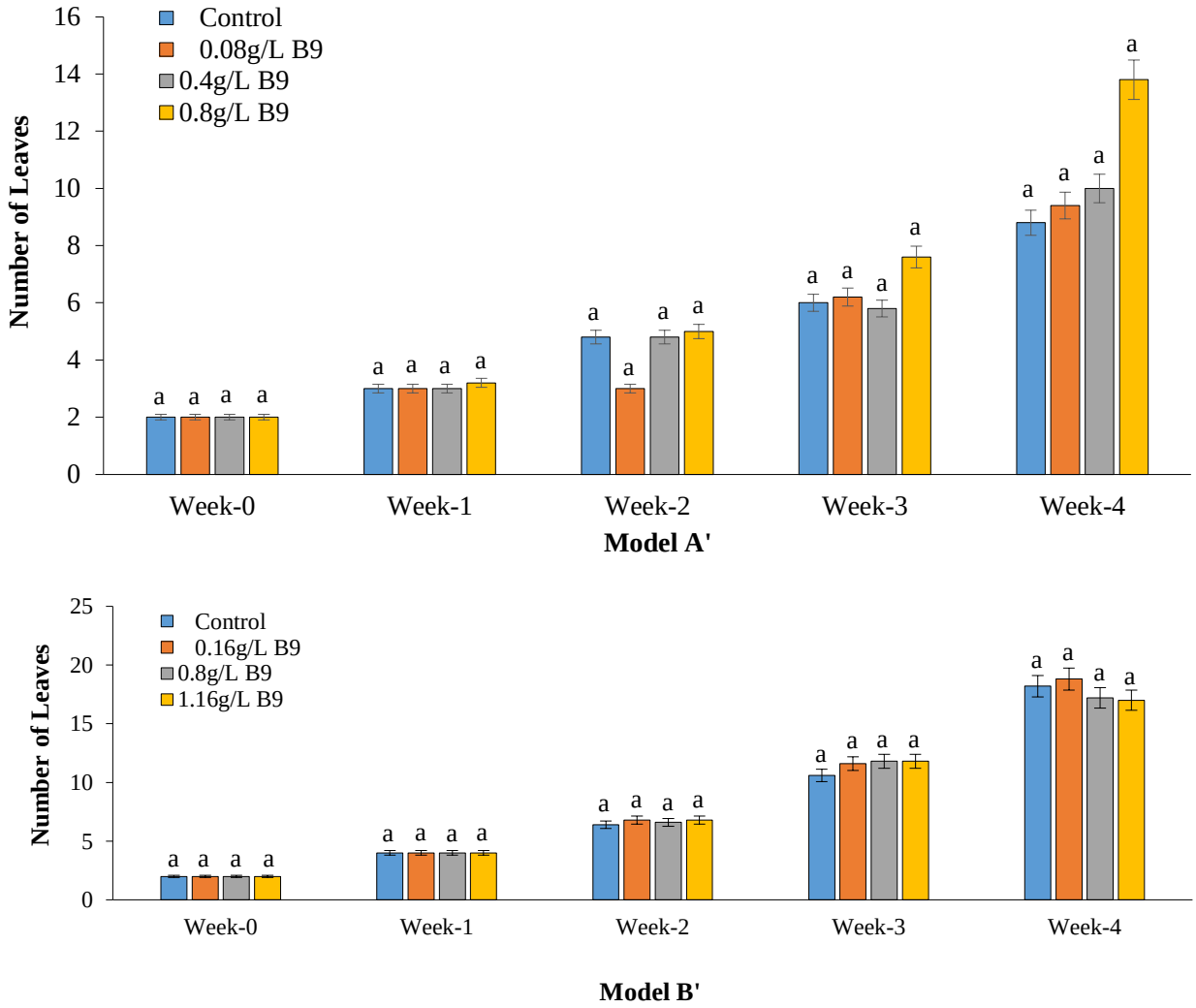


Figure 2.13 Effects of B-NINE (B9 in figure) inhibitor on leaf size in width.

Size of leaves

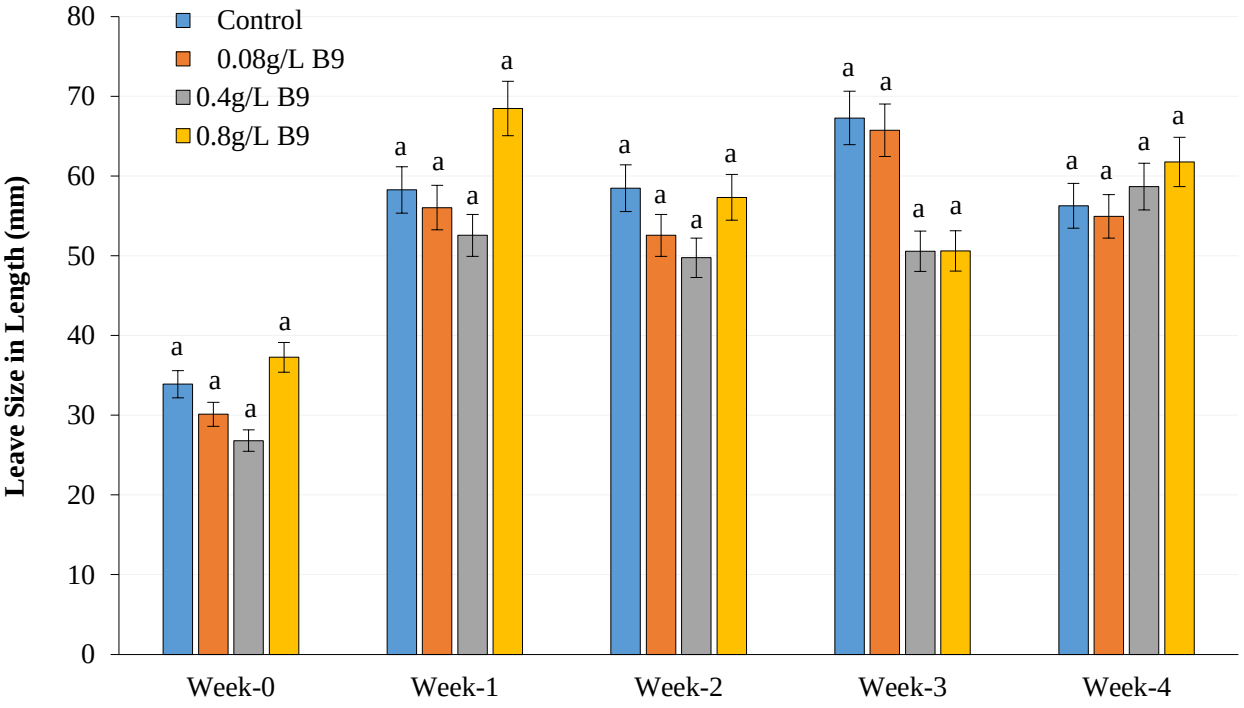
The size in length

In model A', the effects of B-NINE on leaf size in length were not clear for example the plants that received 0.8g/L B-NINE had longer leaves than the plants that received lower concentration of B-NINE and the control plants. And the plants received 0.08g/L and 0.4g/L B-NINE had shorter leaves than the control plants (Figure 2.14, model A'). And there were not significant differences among all treatments in all weeks by statistical analysis.

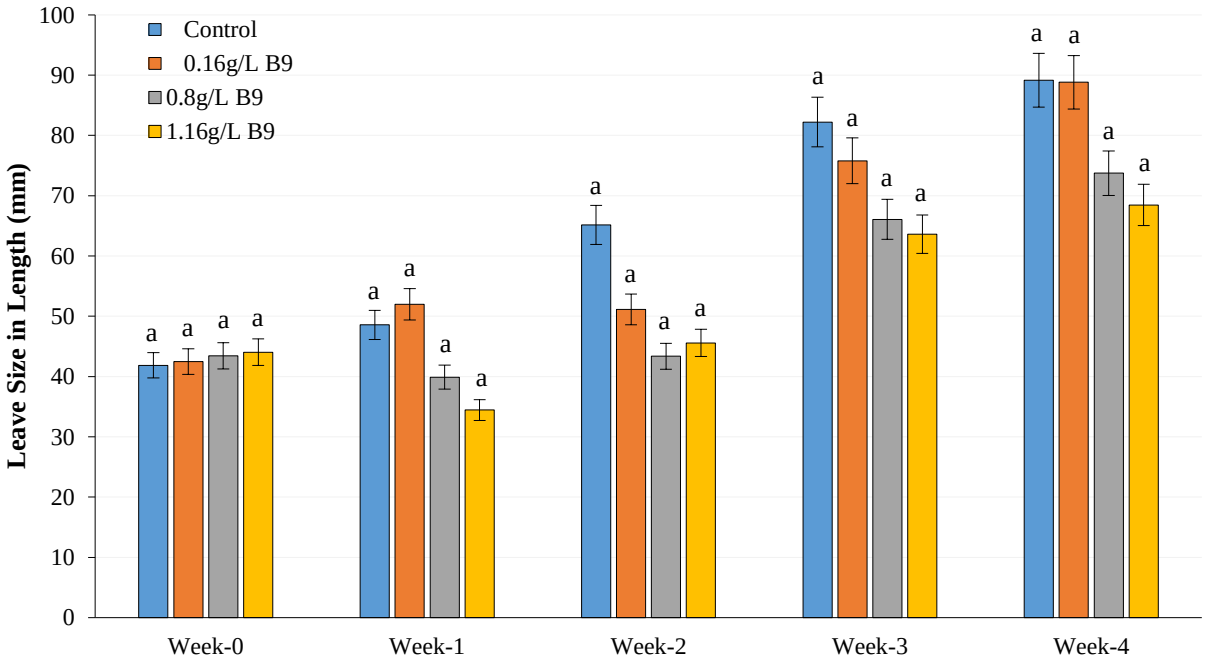
In model B', the plants that received B-NINE of any concentration were inhibited the length of the leaves at week-3 and week-4. The plants received higher concentration of B-NINE had shorter length than the plants received lower concentration of B-NINE and the control plants. However, there were not significant differences by statistical analysis among all treatments (Figure 2.14, model B').

The size in width

In model A', the effects of B-NINE on leaf's width were also not clear because the leaf's width of plants received 0.8g/L and 1.16g/L B-NINE were inhibited only in week-3, but they were bigger than the plants received lower concentration of B-NINE and the control plants in other weeks in



Model A'



Model B'

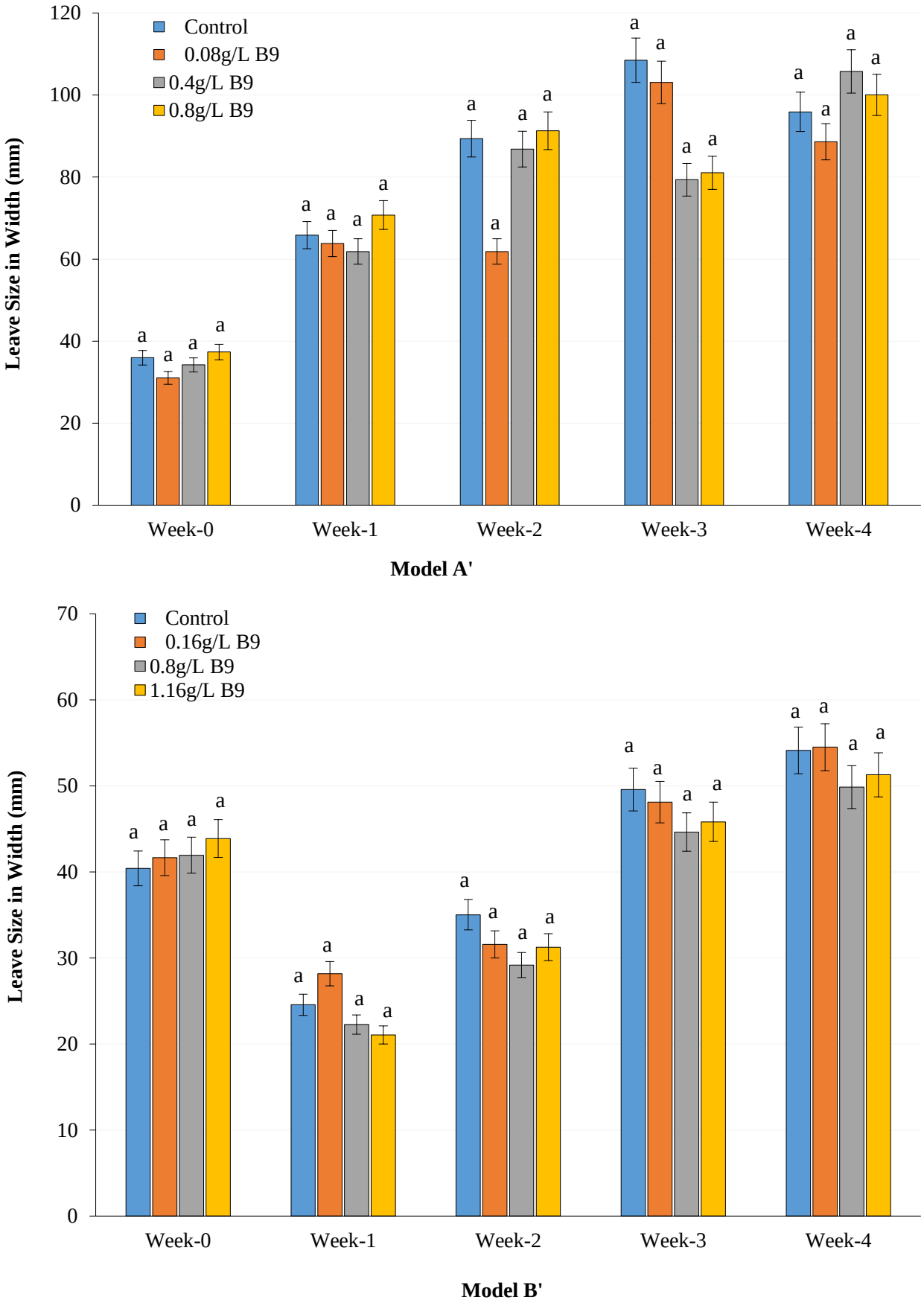


Figure 2.14 Effects of B-NINE (B9 in figure) inhibitor on leaf size in length.

In model B', the plants received 0.8g/L or 1.16g/L B-NINE, their leaves' width were inhibited since the 1st week (week-1) after the experiment started, and these inhibition effects were also observed in the following weeks (week-2 to week-4) (Figure 2.14, model B'). The results in this model showed that high concentration of B-NINE inhibit the leaves' width grow of the plants. However, there were not significant differences between plants received B-NINE and the plants did not receive B-NINE.

The effects of B-NINE on common bean cultivar vine Haibushi in model B' were always stronger than model A' in both leaves' width and length. This might indicate that strong concentration of B-NINE effects to inhibit leaf size growth. But the effects of B-NINE to inhibit the leaves size in these experiments could not concluded because there were not significant differences by statistical analysis.

3.2.2 The reproductive organs deployment

Number of flower

In model A', the result showed that the plants in 0.4g/L B-NINE treatment had the largest amount of flower number among all treatments. And all the plants treated with B-NINE promoted flower induction in comparison to control plants (Figure 2.14a, model A'). But there were not significant differences by statistical analysis.

In model B', B-NINE promoted the number of flower inducing. The plants treated with B-NINE at any concentration had more flower numbers than the plants did not receive the B-NINE. The plants received higher concentration of B-NINE promoted more number of flowers induction gradually (Figure 2.14a, model B'). But there were not significant differences by statistical analysis.

From these two experimental models, the function of B-NINE is opposite. B-NINE is reported as plant growth inhibitor, but the result in this part showed that B-NINE promote the induction of flower numbers of common vine cultivar Haibushi. It will be good for agriculture field if the B-NINE promotes the inducing of flower numbers.

Seed weight

In model A', the plants received 0.08g/L had the heaviest seed weight among all treatments. But plants received higher concentration of B-NINE reduced the seed weight compared to the seeds weight of the control plants. From this result, the higher concentration of B-NINE inhibited the seed weight. But there were not significant differences by statistical analysis (Figure 2.14b, model A').

In model B', the plants received 0.16g/L B-NINE seemed slightly promoted seed weight compared to the control plants, but the higher concentration of B-NINE from 0.8g/L inhibited the seed weight (Figure 2.14b, model B'). However, there were not significant differences by the statistical analysis.

The results of these two model experiments showed that the higher concentration of B-NINE inhibited the seed weight of common bean cultivar Haibushi. Model B' showed stronger inhibition than in model A'. But this effect did not proof by statistical analysis.

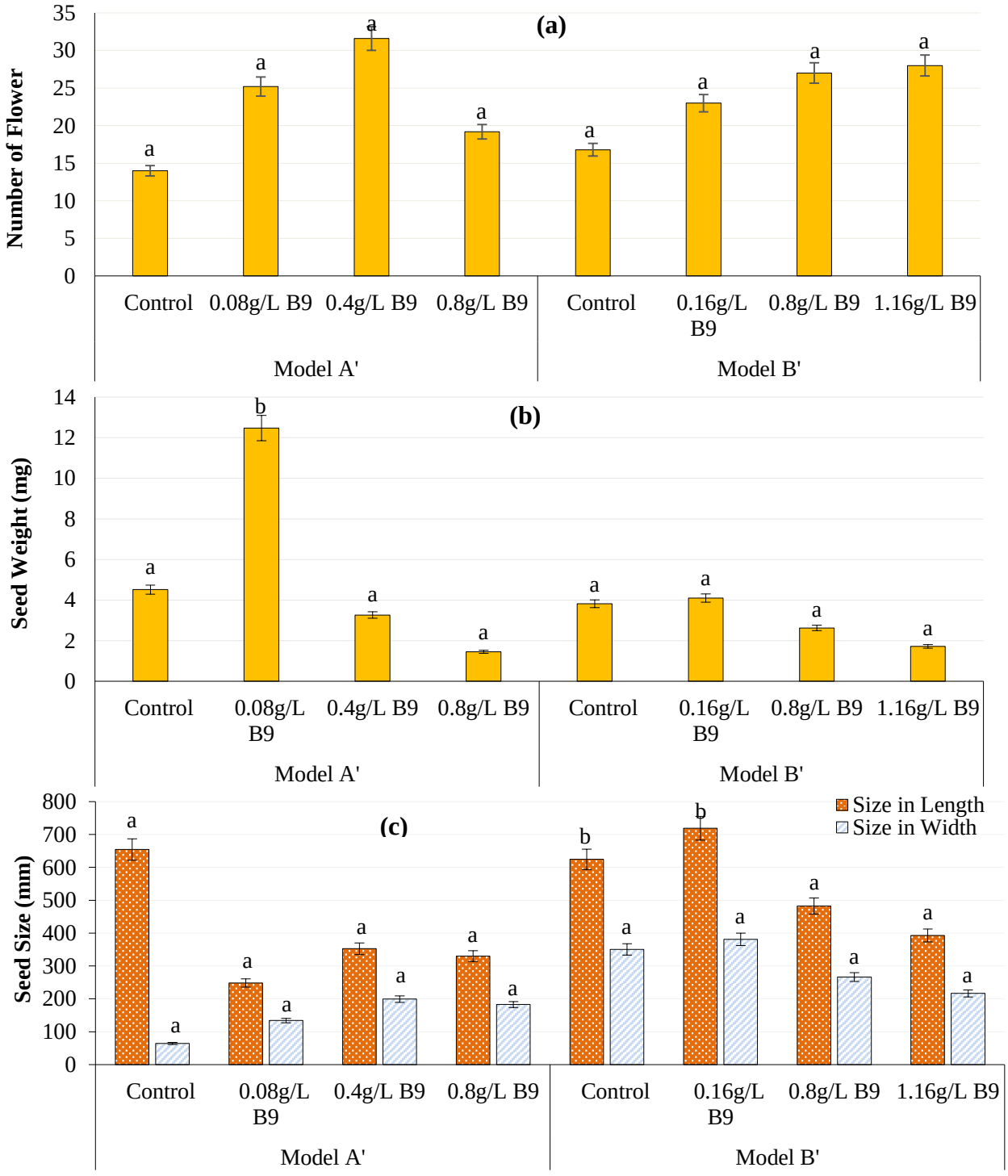


Figure 2.15 The reproductive organs development, (a) flower, (b) seed weight, and (c) seed size.

Seed size

In model A', seed size in both seed length and seed width of the plants received B-NINE treatment were inhibited compared to the seeds of the control plants. But plants received higher B-NINE concentration from 0.4g/L had bigger seeds size than the plant received 0.08g/L (Figure 2.14c, model A'). The seed size in length of plants received B-NINE were significant differences from those in the control plants.

In model B', seed size in both seed length and seed width of the plants received B-NINE were inhibited, except the seed size in length of the plants received 0.16g/L B-NINE, compared to the seed of the control plants. The seeds of plants received high concentration of B-NINE were smaller than the seeds of plants that did not receive B-NINE treatment (Figure 2.14c, model B'). In this experimental model, there were significant differences between seed length received high concentration of B-NINE from 0.8g/L and the seed length of the control plants. The results from model A' and model B' confirmed that B-NINE inhibits the seed size of common bean cultivar vine Haibushi and there were a significant differences by statistical analysis in seed length between the plants received B-NINE and the control plants.

2.4 Conclusion

Plant growth promoting hormone, GA_3: the experiments in both models A and model B showed effects of GA_3 on stem as well as internodes elongation well because the GA_3 at high concentration promote stem and internodes elongation significant longer compared to the plants received low concentration of GA_3. The effects of GA_3 on number of branches producing were also good to introduced to students because high concentration of GA_3 promoted the number of branches producing with significant differences by statistical analysis. The number of leaves and the size of leaves were also promoted by GA_3 spraying, but there was not significant differences so it might be difficult to apply for biological class in high school. The effects of GA_3 on reproductive organ development were not clear through these experiments and there were not significant differences by statistical test analysis, so it might not be appropriate to apply for biological class in high schools. Finally, we can conclude that the experimental methods in model A, the GA_3 source, and the common vine-less cultivar Morocco are suitable to apply for biology experimental class in Cambodia to study their effects on stem and internode elongation. Temperature might effected the results of this experiment, too hot and too cold temperature is not good condition for common bean vine-less cultivar Morocco to grow. The experiment should be done in the temperature from 21^{o}C to 30^{o}C and GA_3 concentration should be started from 5mg/L.

Plant growth inhibitor hormone, B-NINE: the experiments in both models A' and model B' showed good results that B-NINE had strong inhibition on stem and internodes of common bean vine cultivar Haibushi and there were significant differences between plants received high concentration of B-NINE and the plants received low concentration of B-NINE. The effects of B-NINE on number of leaves, number of branches, leaf size, and other reproductive organs were not clear and there were not significant differences by statistical analysis. From these experiments, we can conclude that the experimental methods in model B', the B-NINE source, and the common bean vine cultivar Haibushi are good for application for biological experimental class in high school in Cambodia.

Chapter 3

The Genetics of Mendel's 2nd Law and 3rd Law in Stem Length, Stem Color and Flower Color in Common Bean Cultivar (*Phaseolus vulgaris* L.)

3.1 Introduction

Gregor J. Mendel, an Austrian monk, introduced a theory of inheritance based on his experimental work with pea plants. Mendel believed that heredity is the result of discrete alleles (genes). Two alleles that form the pair for a trait are called homozygous and if the two genes are different are called heterozygous for the trait (Mendel 1865; Bateson W., 1901). Through his research, he defined three laws of inheritances. Mendel's 1st law which is law of dominance states that when crossing between two organisms of different traits, each offspring exhibits the trait of one parent only, the dominant factor is present in an individual. Mendel's 2nd law, the law of segregation states that for any trait, each parent's pairing of genes (alleles) split during meiosis, and the allele from each parent pair up to form a trait of an individual. The F_2 populations are segregated with the 3:1 ratio of dominant and recessive traits. Mendel's 3rd law, the law of independent assortment states that the alleles of two (or more) different genes get sorted into gametes independently of one another and the F_2 populations are segregated the ratio 9:3:3:1. Mendel's law of heredity including the incomplete dominant characteristic is studied in the 11th grade biology textbook (Yihoop *et al*., 2016). However, there are not experimental practices to study Mendel's law of heredity in high school in Cambodia. In this research, I crossed common bean cultivars to study Mendel's 2nd and 3rd law.

3.2 Materials and methods

3.2.1 Plant materials

This crossing was attempted between commercial vine common beans "Haibushi cultivar" as the male parent (pollen) and a vine-less "Morocco cultivar" as the female parent (ovule). Haibushi cultivar has a long-purple stem and red color flower, and Morocco cultivar has a short-green stem and white color flower.

3.2.2 Crossing method

This crossing method followed to Temple *et al* (1987) who did the emasculation on their common bean pollination. They established two ways of human made pollination: 1) the pollination without emasculate and 2) the pollination emasculated. In this study, thus, choose the technique (2) for crossing. First, a flower bud (before flower opened stage) was selected from the Morocco cultivar as a female parent and an opened flower selected from Haibushi cultivar as a male parent (Figure

3.1a). The female parent was emasculated by gently hold the flower between the thumb and index finger, then used forceps' tip to open the petal and wing petal and fold it out direction until the keel found (Figure 3.1b). Gently removed the keel which covered till free stigma, then used forceps to cut the all stamens from the part around stigma (Figure 3.1c). And then used pressure plastic bottle shoot with cleaning water to spray on the stigma to ensure that all pollen removed. For the male parent flower, used a forceps' tip to collect the stamen and then attached on female emasculated stigma (Figure 3.1f). Finally, fold female's petal to close the crossed pistil, then covered pistil with a plastic sticker to keep the moisture inside the flower for fertilization support and to protect the crossing flower from insects attaching (Figure 3.1g). Finally, the crossed flower was labelled (Figure 3.1h).

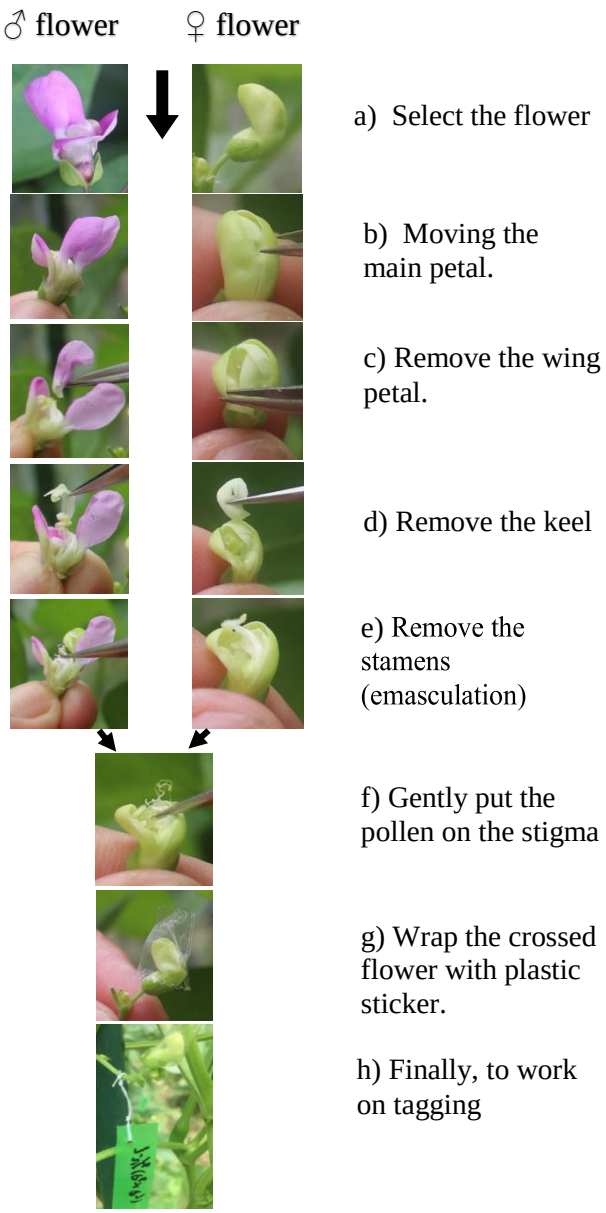


Figure 3.1 crossing procedure and methods**.**

3.2.3 F_1 and F_2 producing

F_1 seeds obtained from the crossing were kept for a day under sunlight for seed dormancy. Dried F_1 seeds were sown in the small plastic pods (pod size 6cm in height, 9cm in radius) with Japanese commercial fertilizer soil (Hana to Yasai not Tchi) for two weeks, then the young plants were transplanted to the big plastic pods (pod size 90 x 30 x 30 in centimetre) Seven F_1 plants were cultivated and kept for self-fertilization during early July 2020. During the middle of August, many F_1's were appeared, but unfortunately all fruits did not consist of the seeds. This failing result was due to the highest temperature season in August (30^0C- 40^0C), which may cause pollen to dead on the fertilizing stage, and/or the fertilized seed was not developed. However, the latest F_1 fruits consisted of more than 350 seeds due to the temperature was decreased (a round 28^0C-30^0C). F_1 seeds were collected and kept under sunlight for a day to make seed dormancy before sewing. In early October, all seeds obtained from those seven F_1 plants sown in the small plastic pods, and the young plants were transplanted into

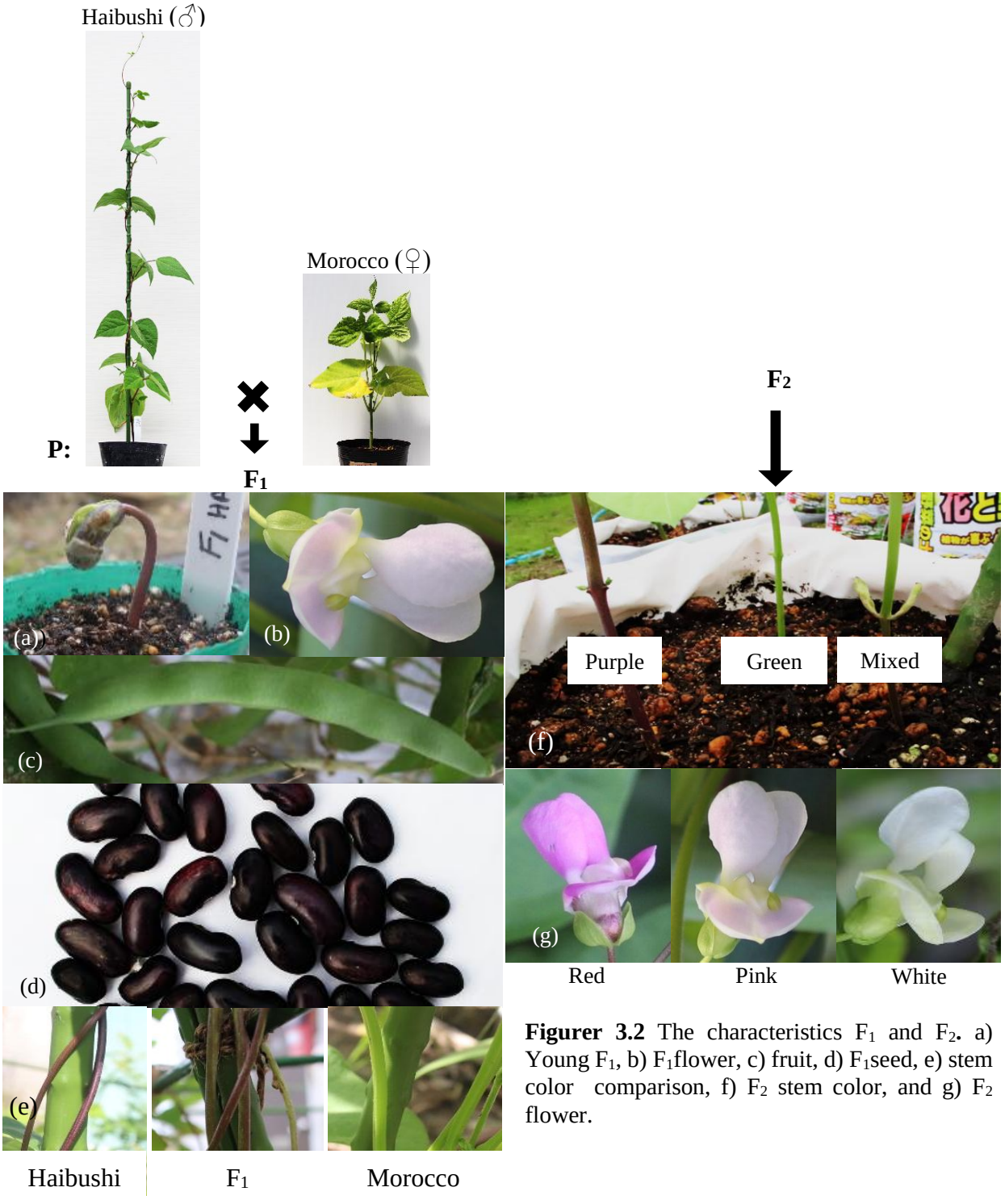
117 bags of Japanese commercial (one bag were three plants) and kept in a plastic green house to grow and induce flower.

3.2.3 Statistical analysis

The statistical test, probabilities of chi-square was calculated by free online software for calculation for the chi-square test by Kristopher J. Preacher ©2010-2020.

3.3 Result and discussion

3.3.1 F_1 plants' characteristics



Figurer 3.2 The characteristics F_1 and F_2**.** a) Young F_1, b) F_1flower, c) fruit, d) F_1seed, e) stem color comparison, f) F_2 stem color, and g) F_2 flower.

The cross between "Haibushi cultivar" with long and purple stem and "Morocco cultivar" with short and green stem, all F_1 offspring were all long and mixed color stem plants (Figure 3.2e, middle). The normal stem characteristic of F_1 plants was complete dominant on short stem characteristics because all F_1 progenies showed long stem. The mixed stem color characteristics of F_1 plants showed the incomplete dominant of purple stem and green stem. The cross between red flower Haibushi cultivar and white flower Morocco cultivar, F_1 offspring were all pink flower plants (Figure 3.2b). Pink flower characteristics is an incomplete dominant between red and white flower characteristics.

3.3.2 Incomplete dominant characteristics (F_2)

Table 3.1 Incomplete dominance in stem color characteristic of F_2 segregation**.**

F_1 code	observed/ expected	F_2 phenotypes			*n*	chi-square (x^2)	degree of freedom	p-value	proportion
		purple	mixed	green					
14-HAI	observed	7	18	15	40	3.600	2	0.16529	1:2:1
	expected	10	20	10					
11-HAI	observed	9	22	10	41	0.268	2	0.87446	1:2:1
	expected	10.3	20.5	10.3					
12-HAI	observed	5	20	9	34	2.00	2	0.36787	1:2:1
	expected	8.5	17	8.5					
15-HAI	observed	8	30	19	57	4.404	2	0.11060	1:2:1
	expected	14.3	28.5	14.3					
8-HAI	observed	10	24	20	54	4.370	2	0.11245	1:2:1
	expected	13.5	27	13.5					
10-HAI	observed	12	12	13	37	4.622	2	0.09918	1:2:1
	expected	9.25	18.5	9.25					

Table 3.2 Incomplete dominance in flower color characteristic of F_2 segregation.

F_1 code	observed/ expected	F_2 phenotypes			*n*	chi-square (x^2)	degree of freedom	p-value	proportion
		red	Pink	white					
14-HAI	observed	10	18	12	40	0.556	2	0.75747	1:2:1
	expected	10	20	10					
11-HAI	observed	6	27	8	41	4.317	2	0.11549	1:2:1
	expected	10.25	20.5	10.25					
12-HAI	observed	4	21	9	34	3.353	2	0.18703	1:2:1
	expected	8.5	17	8.5					
15-HAI	observed	13	29	15	57	0.158	2	0.92409	1:2:1
	expected	14.25	28.5	14.25					
8-HAI	observed	16	19	19	54	5.074	2	0.0791	1:2:1
	expected	13.5	27	13.5					
10-HAI	observed	11	18	8	37	0.514	2	0.77356	1:2:1
	expected	9.25	18.5	9.25					

Table 3.1 showed that F_2 population obtained from all F_1 plants had the proportion ratio, 1:2:1 (purple: mixed color: green) stem plants. Through chi-square test there was not different between expected number and observed number of F_2 population because all *p*-values are bigger than 0.05. Table 3.2 also showed that F_2 population obtained from the cross between red flower Haibushi and white flower Morocco had the ratio, 1:2:1 (red: pink: white) flower plants. The F_2 population results can confirm that the stem color characteristics and flower color characteristics studied in these experiments are incomplete dominant inheritance characteristics. The proportion ratio of F_2 population can be used to explain the inheritance characteristics in Mendel's 2nd law.

3.3.3 Stem length characteristics (F_2)

In the F_2 population of stem length, although population of 12-HAI and 15-HAI were segregated as 3:1 (Normal or long stem; vine-less or short stem), the segregated proportion of populations of 14-HAI, 11-HAI, and 8-HAI were deviated from the ration of 3:1 by chi-square test (Table 3.3).

Table 3.3 Chi-square test of F_2 stem length segregation.

F_1 code	observed expected	F_2 phenotypes		*n*	chi-square (x^2)	degree of freedom	*p*-value
		Tall	short				
14-HAI	observed	23	17	40	6.533	1	0.0105
	expected	30	10				
11-HAI	observed	23	18	41	7.813	1	0.0051
	expected	30.75	10.25				
12-HAI	observed	24	10	34	0.353	1	0.55245
	expected	25.5	8.5				
15-HAI	observed	39	18	57	1.316	1	0.25135
	expected	42.75	14.25				
8-HAI	observed	34	20	54	4.173	1	0.041
	expected	40.5	13.5				

3.3.4 Epitasis on Stem length characteristics (F_2)

In genetics of biosynthesis product, because many precursor products were produced by each enzyme regulated each gene, same recessive phenotype showed by alleles of different gene. GA_3 was also produced through flow of GA biosynthesis. When the gene locus of precursor product enzyme gene changed to null or leaky alleles and the plants with homozygous of these alleles, plant phenotype showed dwarf or vine-less; when different two or more gene loci of precursor product enzyme genes changed to null or leaky alleles and the plants with both homozygous of alleles in each loci, phenotype showed the same as dwarf or vine-less. So, hypothesis of these three populations' vine-less, 11-HAI, 14-HAI, and 8-HAI, were changed 3:1 to 9:7, which proportion based on 9:3:3:1 of dihybrid genetics. In the chi-square test between observed number and expected number calculated by 9:7 showed no statistical differences presented (Table 3.4). These

results suggested that the seeds of vine-less parents, cv. Morocco with vine-less character might not be uniform of vine-less genotype, but two genotypes. The grand-parents of F_2 populations of 12-HAI and 15-HAI had genotype of recessive homozygous in a gene. This genotype designated as d_1d_1. However, the F_2 population of 14-HAI, 11-HAI and 8-HAI showed epistasis segregation as 9:7=Normal and vine-less, the genotype of grandparent with vine-less phenotype was affected two vine-less gene loci, which genotype was $d_1d_1d_2d_2$ or $d_1d_1D_2d_2$. When the genotype of F_1 plants of these three F_2 populations had to be $D_1d_1D_2d_2$, F_2 populations could segregate as 9:7. Because p-value of these segregated as 9:7 showed high value, vine-less. The proportion ratio of 9:7 inheritance in this study can be used to explain Mendel's 3rd law.

Table 3.4 The proportion 9:7 of stem length segregation.

F_1 code	**observed expected**	**F_2 phenotypes**		***n***	**chi-square (x^2)**	**degree of freedom**	***p*-value**	**proportion**
		tall	**short**					
14-HAI	observed	23	17	40	0.025	1	0.87338	9:7
	expected	22.5	17.5					
11-HAI	observed	23	18	41	0	1	0.9843	9:7
	expected	23.0625	17.9375					
8-HAI	observed	34	20	54	0.989	1	0.32003	9:7
	expected	30.375	23.625					
total	**expected**	**80**	**55**	**135**	**0.497**	**1**	**0.481**	**9:7**
	observed	**75.9375**	**59.0625**					

3.4 Conclusion

The experiments by crossing common bean cultivar Haibushi and cultivar Morocco are good to introduced to biology class in high school level. The results of the experiments are good references to explain students about Mendel's 2nd and 3rd laws of inheritance. The incomplete dominant characteristics of stem color and flower color are used to explain Mendel's 2nd law of inheritance. The proportion ratio 3:1 of long and short stem plants can be used to explain Mendel's 2nd law of inheritance and the ratio 9:7 is new inheritance characteristics to apply for biology education in Cambodia to explain Mendel's 3rd law. This experiment takes long time so it should be applied as a research activities for students.

Chapter 4

Linkage Analysis of Incomplete Dominant Pair, Flower and Stem Color in Common Bean (*Phaseolus vulgaris* L.) by Direct Recombination Value Estimation

4.1 Introduction

The genetic linkage found by Thomas H. Morgan (1911) used the backcross population of a fruit fly. Production of cross progeny of fruit fly was tended to be easier because it has diphenotype of male and female, and there was no self-fertilization occurred. In high school, using the fruit fly for the genetic study is difficult because teachers have to keep parents' strain by feeding. It is easy to use self-fertilization in a plant to produce the population, but in the genetic linkage, calculating the recombination value is very difficult due to the dominance phenotype involved homozygous and heterozygous, and it is also challenging to separate the homozygous and heterozygous on observation.

Incomplete dominance is described in a high school textbook, and students learn to know that the hetero type should be an intermediate characteristic, and phenotypes were easy to estimate into genotype. Allard (1956) reported the method to estimate recombination value in heredity in the F_2 population. However, his formula is too difficult for high school students to understand. In the linked pairs of different incomplete dominance character, most F_2 phenotype could be estimated into linked genotypes simply. In this study, linked two incomplete dominance characteristics between stem color and flower color were found in F_2 populations of common bean, and this segregated proportions were deviated from the proportion estimated by Mendel's 3^{rd} law. Recombinant value was calculated by direct methods and results of chi-square test between reconstructed expected value depended on recombinant value and observed in most F_2 population did not show statistical differences. Thus to use the common bean with incomplete dominance characteristics, the genetic linkage for education material was developed for experimental biology class in high school education.

4.2 The aim of the study

This study is aim to analysis of the genetic linkage of incomplete dominant pair, flower and stem color in common bean (*Phaseolus vulgaris* L.) by direct recombination value estimation, applicable for high school education.

4.3 Materials and methods

4.3.1 Plant materials and F_2 population producing

The cross between common bean cultivars Haibushi and Morocco in chapter 3 was used to study about incomplete dominant pair, stem color and flower color. Haibushi cultivar has purple a stem and red color flower, and Morocco cultivar has a green stem and white color flower. F_1 and F_2 plants cultivation were already explained in chapter 3.

4.3.2 Methods for calculation expected genotypes and phenotypes

For solving the genetic linkage, the recombination rate (r) was calculated from observing data of F_2 characteristic segregation by using excel. In this calculation, I noted that st^{pig} is purple stem color allele, fl^{pig} is red flower color

allele, g is green stem color allele, and w is white flower color allele.

4.3.2.1 Calculation recombination rate *(r)*

At first, to estimate linked genotype from observed phenotype, then count all four linked genotype.

$r = \frac{Estimated\ recombination\ chromosome}{Total\ estimated\ observ\ chromosome\ number}$. In this study, F_1 genotype of $\frac{st^{pig} - fl^{pig}}{g - w}$ produced four linked genotypes on chromosome in meiosis, which parental genotype $st^{pig} - fl^{pig}$ and $g - w$, and recombinant genotypes $st^{pig} - w$ and $g - fl^{pig}$. Therefore,

$$r = \frac{(st^{pig} - w) + (g - fl^{pig})}{(st^{pig} - fl^{pig}) + (g - w) + (st^{pig} - w) + (g - fl^{pig})}.$$

4.3.2.2 Calculation of F_2 gene proportion value

To reconstructed expected value, the theoretical proportion of all four chromosome genotype $st^{pig} - fl^{pig}$, $g - w$, $st^{pig} - w$, and $g - fl^{pig}$ equal 1. Since genotype of $st^{pig} - fl^{pig} =$ genotype of $g - w$ and gene of $st^{pig} - w =$ gene of $g - fl^{pig} = \frac{r}{2}$. Thus, gene of $st^{pig} - fl^{pig} = \frac{1-r}{2}$.

4.3.2.3 Calculation of expected F_2 genotypes value

The expect genotype value = ♀genotype × ♂genotype × population. All the expected F_2 genotype value formula show in table 4.1. From the expectation of genotypes calculation, sixteen genotypes and nine phenotypes of F_2 population were produced (Table 4.1 and Table 4.2).

Table 4.1 The formulas used to calculate the expected genotypes of F_2 population.

Note: *r* is the recombination gametes, and *n* is the population size.

Gametes of F_1		***st^{pig}-fl^{pig}***	***st^{pig}-w***	***g-fl^{pig}***	***g-w***
		$\frac{1-r}{2}$	$\frac{r}{2}$	$\frac{r}{2}$	$\frac{1-r}{2}$
st^{pig}-fl^{pig}	$\frac{1-r}{2}$	$\left(\frac{1-r}{2}\right)^2 n$	$\frac{r(1-r)}{4}n$	$\frac{r(1-r)}{4}n$	$\left(\frac{1-r}{2}\right)^2 n$
st^{pig}-w	$\frac{r}{2}$	$\frac{r(1-r)}{4}n$	$\left(\frac{r}{2}\right)^2 n$	$\left(\frac{r}{2}\right)^2 n$	$\frac{r(1-r)}{4}n$
g-fl^{pig}	$\frac{r}{2}$	$\frac{r(1-r)}{4}n$	$\left(\frac{r}{2}\right)^2 n$	$\left(\frac{r}{2}\right)^2 n$	$\frac{r(1-r)}{4}n$
g-w	$\frac{1-r}{2}$	$\left(\frac{1-r}{2}\right)^2 n$	$\frac{r(1-r)}{4}n$	$\frac{r(1-r)}{4}n$	$\left(\frac{1-r}{2}\right)^2 n$

Table 4.2 The expectation of F_2 phenotypes and genotypes.

		♀ Pistil			
F_1 gametes		$\boldsymbol{st^{pig}-fl^{pig}}$	$\boldsymbol{st^{pig}-w}$	$\boldsymbol{g-fl^{pig}}$	$\boldsymbol{g-w}$
♂ Pollen	$\boldsymbol{st^{pig}-fl^{pig}}$	$\frac{st^{pig}-fl^{pig}}{st^{pig}-fl^{pig}}$ Purple-Red	$\frac{st^{pig}-w}{st^{pig}-flpig}$ Purple-Pink	$\frac{g-fl^{pig}}{st^{pig}-flpig}$ Mixed-Red	$\frac{g-w}{st^{pig}-flpig}$ Mixed-Pink
	$\boldsymbol{st^{pig}-w}$	$\frac{st^{pig}-fl^{pig}}{st^{pig}-w}$ 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
	$\boldsymbol{g-fl^{pig}}$	$\frac{st^{pig}-fl^{pig}}{g-fl^{pig}}$ 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
	$\boldsymbol{g-w}$	$\frac{st^{pig}-fl^{pig}}{g-w}$ 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

The expectation of F_2 phenotypes and genotypes from calculation in table 4.1 as following

- Purple-Red $\frac{st^{pig}-fl^{pig}}{st^{pig}-fl^{pig}}=\frac{(1-r)^2}{4}n$.
- 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-Red $\frac{st^{pig}-fl^{pig}}{g-fl^{pig}}+\frac{g-fl^{pig}}{st^{pig}-fl^{pig}}=\frac{r(1-r)}{2}n$.
- Mixed-Pink $\frac{st^{pig}-fl^{pig}}{g-w}+\frac{g-w}{st^{pig}-fl^{pig}}+\frac{st^{pig}-w}{g-fl^{pig}}+\frac{g-fl^{pig}}{st^{pig}-w}=\frac{r^2+(1-r)^2}{2}n$.
- Mixed-White $\frac{st^{pig}-w}{g-w}+\frac{g-w}{st^{pig}-w}=\frac{r(1-r)}{2}n$.
- Green-Red $\frac{g-fl^{pig}}{g-fl^{pig}}=\frac{r^2}{4}n$.
- Green-Pink $\frac{g-fl^{pig}}{g-w}+\frac{g-w}{g-fl^{pig}}=\frac{r(1-r)}{2}n$.

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

Chi-square test between observed phenotype and reconstructed expected value by *r* obtained from direct methods.

$$Chi-square=\sum\frac{(O-E)^2}{E}$$

The degree of freedom was category – estimated step (9-2=7).

4.4 Result and discussion

In this study, Haibushi cultivar have purple stem and red flower was crossed with Morocco cultivar have green stem and white flower. All F_1 plants obtained from this cross were all mixed stem color and pink flower color plants. The F_2 population was segregated into nine different phenotypes, including purple stem-red flower, purple stem-pink flower, mixed color stem-red flower, mixed color stem-pink flower, mixed color stem-pink flower, purple stem-white flower, mixed stem-white flower, green stem-red flower, green stem-pink flower, and green stem-white flower plants (Table 4.2).

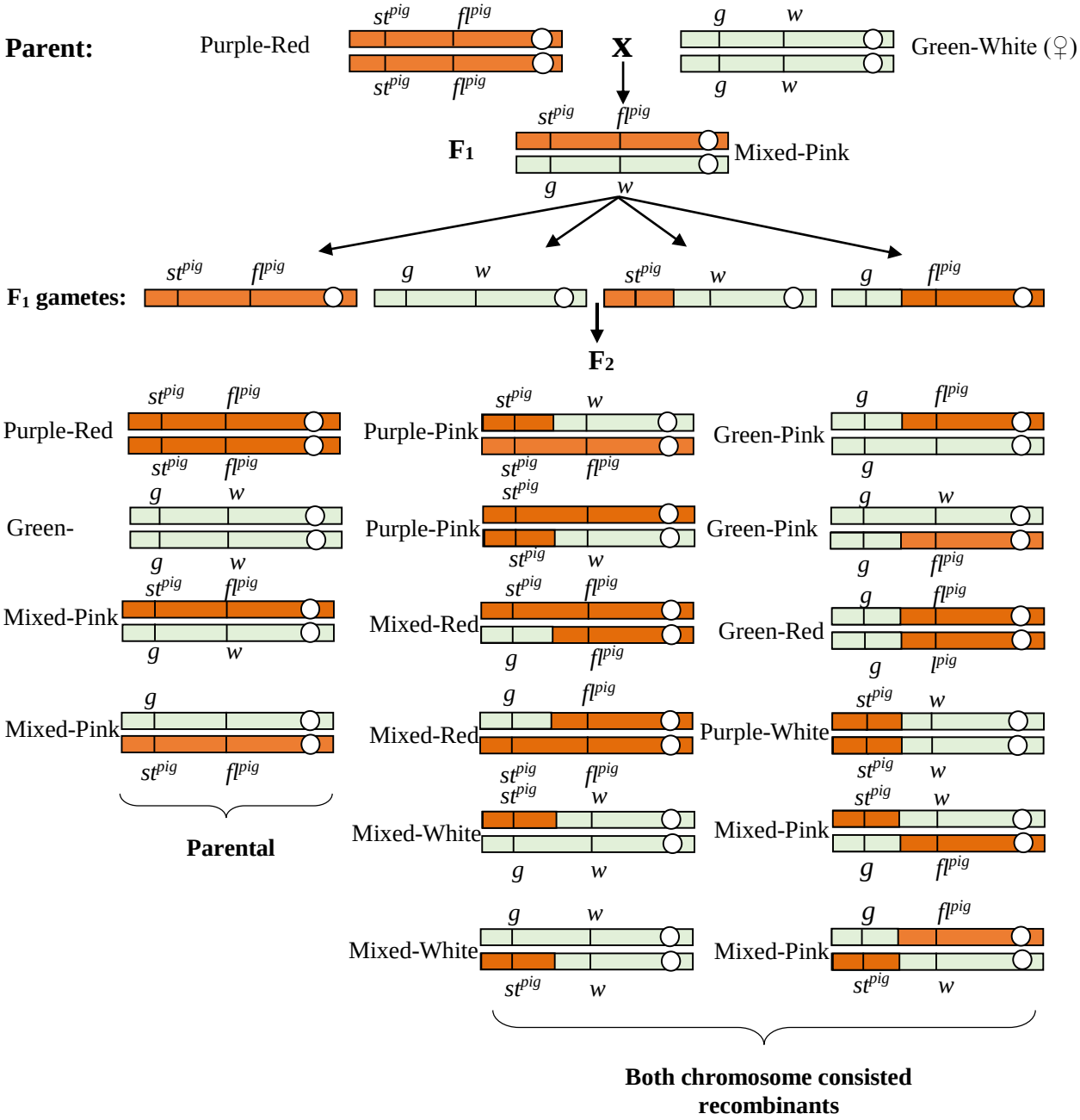
The genotype of the F_1 plant with mixed color stem and the pink flower was estimated to $\frac{st^{pig}-fl^{pig}}{g-w}$, and in meiosis, this F_1 plant produces four different gametes as indicated in Table 4.1. Furthermore, the phenotypes and genotypes of the F_2 population also showed by chromosome images in the Figure 4.1, which explained the genetic linkage of stem color and flower color in this study.

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 were inputted in to excel database, and each plants data observed in both colors characteristics were changed to estimated genotype combination on the chromosome. From these estimated genotype combination on the chromosome, *r*-value were calculated by total of recombinant chromosomes/total chromosomes. Each *r*-value calculated of each population were 0.2125, 0.1029, 0.0965, 0.1204, and 0.2162, respectively. Chi-square test between observed value and reconstructed expected value using *r* value indicated that only observed value population 11-HAI was deviated from expected value and other populations did not have significant differences, so these recombinant values were acceptable.

Two weak point of these calculation were imaged; (1) to change from phenotype to genotype, 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, because number of plants in each population was not so many, the expected value of F_2

plants with both different recombinant chromosome pairs, which calculated by $\frac{r^2}{2}n$ was about 1 or 2 when r=0.1. So, students could ignore these value. If *r*-value is too big or number of F_2 plants is also too big, consideration is needed. (2) This methods *r*-value was different by each population, teachers have to explain the student of this reason. But the differences depended on observed value. So, when F_2 populations obtained from same F_1 plants cultivated repeat, looked like the same *r*-value may present. This results of linked incompletely dominance characters will be good materials to use in high school genetic education because students could image genotype on a chromosome from phenotype. Because mostly backcross population were used in linkage study, student have understood linkage calculation as number of individuals with recombinant phenotype per total number of individuals and there were no chromosome images.

Common bean stem color and flower color results based on F_2 population results. Thus, it was very important to have chromosome image for students in estimation of genotypes from phenotype as indicated in Figure 4.1. The results of gene linkage study of stem color and flower color characteristics in this study were significant differences between the expected number and observed number of F_2 plants because all *p*-values>0.05 in the chi-square test (Table 4.3).



Figurer 4.1 Genetic linkage mapping of F_2 population.

Table 4.3 Recombination rate between stem color and flower color of F_2 segregation, calculated by chi-square at 7 degree of freedom.

F_1 code	observed expected	F_2 phenotypes									*n*	*r* (%)	chi-square x^2	degree of freedom	*p*-value
		purple red	purple pink	mixed red	mixed pink	purple white	mixed white	green red	green pink	green white					
11-HAI	observed	1	8	5	16	0	1	0	3	7	41	0.2125	14.494	7	0.01<P<0.05
	expected	6.36	3.43	3.43	13.64	0.46	3.43	0.46	3.43	6.36					
12-HAI	observed	2	3	2	17	0	1	0	1	8	34	0.1029	5.636	7	0.5<P<0.7
	expected	6.84	1.57	1.57	13.86	0	1.57	0.09	1.57	6.84					
15-HAI	observed	7	1	6	24	0	0	0	4	15	57	0.0965	12.364	7	0.05<P<0.1
	expected	11.63	2.48	2.48	23.53	0.13	2.48	0.13	2.48	11.63					
8-HAI	observed	9	1	7	15	0	2	0	3	17	54	0.1204	14.031	7	0.05<P<0.1
	expected	10.45	2.86	2.86	21.28	0.20	2.86	0.20	2.86	10.45					
10-HAI	observed	7	5	3	8	0	1	1	5	7	37	0.2162	6.93	7	0.3<P<0.5
	expected	5.68	3.14	3.14	12.23	0.43	3.14	0.43	3.14	5.68					
total	**observed**	**26**	**18**	**23**	**80**	**0**	**5**	**1**	**16**	**54**	**223**	**0.1435**	**25.556**	**7**	**0<p<0.001**
	expected	**41**	**13.5**	**13.5**	**84.5**	**1.31**	**13.5**	**1.31**	**13.5**	**41**					

4.5 Conclusion

The method to calculate the expected number of F_2 genotypes and phenotypes introduced in this study is useful for high school students and teacher trainees at National Institute of Education in Cambodia for their study about genetic linkage. The experimental result in this chapter is a good reference to teach students about genetic linkage because this content has not yet included in biology textbook in Cambodia. The chromosome mapping is good to explain students how the linkage gene inherited from parents to offspring.

Chapter 5

Linkage Analysis of Incomplete Dominance and Dominance Character Pair, Flower Color and Stem Length, Stem Length and Stem Color, and Stem Color and Flower Color in Common Bean (*Phaseolus vulgaris* L.) by Maximum Likelihood Estimation Method

5.1 Introduction

Thomas H. Morgan (1911) studied the gene linkage using fruit fly. In chapter 4, the linkage analysis of incomplete dominant pair, flower, and stem color was analysed by using direct calculation method. The methods to calculate the recombination value estimation was introduced, and it was concluded that it is good to apply for biology education in high school. The chi-square test statistically analysed the recombinant values of genes in the F_2 population, and there were no differences between the expected number and the observed number because the chi-square test p-values>0.05 in all F_1 plants. The incomplete dominant and dominant character pair, flower color and stem length, and stem length and stem color are different character patterns from the incomplete dominant pair, flower color, and stem color in chapter 4. The methods to calculate the expected number of phenotypes and genotypes in F_2 populations in chapter 4 was the direct estimation. This chapter, Maximum Likelihood Estimation (MLE) method, is used to calculate the expected phenotype and genotype number; and the linkage pairs of stem length and stem color, and stem length and flower color are analysed.

5.2 Materials and methods

5.2.1 Plant materials and F_2 population producing

The cross between common bean cultivars, Haibushi and Morocco in chapter 3 was used to study incomplete dominant and dominant characteristics pairs in stem length and stem color, and stem length and flower color, and stem color and flower color. Haibushi cultivar has a tall-purple stem and red color flower, and Morocco cultivar has a short-green stem and white color flower. F_1 and F_2 plants cultivation were already explained in chapter 3.

5.2.2 Estimation of recombination value (*r*) using chi-square value

In F_2 of incomplete dominance character pairs, directly recombination value calculation methods could be used by estimated genotype pairs from phenotype. However, in F_2 of combination between complete dominance and incomplete dominance pairs, such as the combination between plant height and stem color, genotype could not be estimated from phenotype. The parameter of *r* value calculated the reconstructed expected phenotype. So, the total number of the plant (*n*) was

calculated from each observed value's total number. In stem length and stem color, the reconstructed expected value is divided into nine phenotypic categories. Parental genotype pairs on the chromosome were homozygous of tall-purple or short-green. F_1 genotypes pairs on the chromosome were chromosome combination between tall-purple, and short-green. Gamete combination on the chromosome of F_1 was divided into four chromosomes with genotype pairs, two were chromosomes with parental genotype, and the other two were chromosomes with recombinant genotype. The recombinant value (*r*) is calculated by the total recombinant chromosome divided by all chromosomes in the gamete stage. Total of recombinant pair added the chromosome number with the genotype of tall-green and short-red. The average rate of either genotype was calculated to *r/2* and the Average rate of either parental type was *(1-r)/2*. The expected genotype of F_2 could be calculated as Table 5.1.

Table 5.1 The formula used to calculate the recombination rate of genotypes values of stem length with stem color of F_2 populations by using punnet square.

			♂			
♀	**phenotypes**		**tall-purple**	**tall green**	**short-purple**	**short-green**
			$\frac{1-r}{2}$	$\frac{r}{2}$	$\frac{r}{2}$	$\frac{1-r}{2}$
	tall-purple	$\frac{1-r}{2}$	$\frac{(1-r)^2}{4}$	$\frac{r(1-r)}{4}$	$\frac{r(1-r)}{4}$	$\frac{(1-r)^2}{4}$
	tall-green	$\frac{r}{2}$	$\frac{r(1-r)}{4}$	$\frac{r^2}{4}$	$\frac{r^2}{4}$	$\frac{r(1-r)}{4}$
	short-purple	$\frac{r}{2}$	$\frac{r(1-r)}{4}$	$\frac{r^2}{4}$	$\frac{r^2}{4}$	$\frac{r(1-r)}{4}$
	short-green	$\frac{1-r}{2}$	$\frac{(1-r)^2}{4}$	$\frac{r(1-r)}{4}$	$\frac{r(1-r)}{4}$	$\frac{(1-r)^2}{4}$

The expect genotypes value of stem length with stem color as following

- *E*(Tall-Purple)=(tall-purple*tall-pruple+2*tall-purple*short-purple)*$n = n(1-r)^2/4$.
- *E*(Tall-Miexd)=(2*tall-purple*tall-green+2*tall-purple*short-green+2*short-purple*tall-green)*$n = 2n(1-r+r^2)/4$.
- *E*(Tall-Green) = (tall-green*tall-green+2*tall-green*short-green)*$n = nr(2-r)/4$.
- *E*(Short-Purple) =short-purple*short-purple*$n = nr^2/4$.
- *E*(Short-Mixed) =2*short-purple*short-green*$n = 2nr(1-r)/4$.
- *E*(Short-Green) =short-green*short-green*$n = n(1-r)^2/4$.

Chi-square was calculated similarity between observed value and expected value by the formula of $x^2=\Sigma(\text{observed value-expected value})^2/\text{expected value}$. In using *r* parameter, minimum x^2 value

producing *r* value showed most high *p*-value which was the most similar between observed value and expected value.

5.2.3 Maximum Likelihood Estimate calculation method

In general, Maximum Likelihood Estimate (MLE) methods was used to estimate the recombination rate from the F_2 population. This calculation showed probability. In using the *r* parameter, *r*-value producing the highest probability was most reliable, and chi-square value using this *r*-value could show the minimum value and *p*-value is highest. The probability function of observed genotypes can be express in the form of a multinomial distribution. The probability of frequencies of appearance of observed phenotype number of F_2 as following

$$P(r) = \frac{n!\prod_{i=1}^{m}\left(f_{k_i}\right)^{k_i}}{\prod_{i=1}^{m}k_i!}$$

Where, *m* is the number of observed phenotypes.

n is the population values $(n = k_1 + k_2 + ... + k_m)$.

k_i is the observe value and f_{k_i} : the frequencies phenotype with recombination rate value.

Remark: $(i)\ n! = n(n-1)...2.1$, $(ii)\ \prod_{i=1}^{m}k_i! = k_1!k_2!...!k_m!$ and $(iii)\prod_{i=1}^{m}\left(f_{k_i}\right)^{k_i} = \left(f_{k_1}\right)^{k_1}\left(f_{k_2}\right)^{k_2}....\left(f_{k_m}\right)^{k_m}$.

5.2.3.1 The probability of linkage genotypes between stem length and stem color

The recombination rate *(r)* from the number observed genotypes of F_2 population could be expressed as a probability function. In the F_2 population of couple genotypes between stem length and stem color, the probability of each phenotype was showed by $k_1 \sim k_6$. Measure the number of rolls and consider the probability that the observed value will come out. By table 5.1, we obtain the frequencies phenotype with recombination rate value of stem length with stem color as following

- f_{k_1} : *f*(Tall-Purple)=tall-purple*tall-pruple+2*tall-purple*short-purple $= (1-r^2)/4$.
- f_{k_2} : *f* (Tall-Miexd)=2*tall-purple*tall-green+2*tall-purple*short-green+2*short-purple*tall-green $= 2(1-r+r^2)/4$.
- f_{k_3} : *f*(Tall-Green) = tall-green*tall-green+2*tall-green*short-green $= r(2-r)/4$.
- f_{k_4} : *f*(Short-Purple) =short-purple*short-purple $= r^2/4$.
- f_{k_5} : *f*(Short-Mixed) =2*short-purple*short-green= $2r(1-r)/4$.
- f_{k_6} : *f*(Short-Green) =short-green*short-green= $(1-r)^2/4$.

Therefore, the probability of frequencies of appearance of observed phenotype number of stem length with steam color F_2 as above, in this case $m=6$.

5.2.3.2 The probability of linkage genotypes between stem length and flower color

Calculation methods of *r* value from the coupled data between stem length and flower color were the same as the *r*-value calculation of coupled data between stem length and stem color in F_2. To calculate the expected genotype was below

Table 5.2 The recombination rate of genotypes values of stem length with flower color.

			♂			
♀	**phenotypes**		**tall-red**	**tall white**	**short-red**	**short-white**
			$\frac{1-r}{2}$	$\frac{r}{2}$	$\frac{r}{2}$	$\frac{1-r}{2}$
	tall-red	$\frac{1-r}{2}$	$\frac{(1-r)^2}{4}$	$\frac{r(1-r)}{4}$	$\frac{r(1-r)}{4}$	$\frac{(1-r)^2}{4}$
	tall-white	$\frac{r}{2}$	$\frac{r(1-r)}{4}$	$\frac{r^2}{4}$	$\frac{r^2}{4}$	$\frac{r(1-r)}{4}$
	short-red	$\frac{r}{2}$	$\frac{r(1-r)}{4}$	$\frac{r^2}{4}$	$\frac{r^2}{4}$	$\frac{r(1-r)}{4}$
	short-white	$\frac{1-r}{2}$	$\frac{(1-r)^2}{4}$	$\frac{r(1-r)}{4}$	$\frac{r(1-r)}{4}$	$\frac{(1-r)^2}{4}$

The expected genotype value of stem length with flower color as following

- *E*(tall-red)=tall-red*tall-red+2*tall-red*short-red *$n = n(1-r^2)/4$.
- *E*(tall-pink)=2*tall-red*tall-white+2*tall-red*short-white+2*short-red*tall-white*$n = 2n(1-r+r^2)/4$.
- *E*(tall-white) =tall-white*tall-white+2*tall-white*short-white*$n = nr(2-r)/4$.
- *E*(short-red) =short-red*short-red*$n = nr^2/4$.
- *E*(short-pink) =2*short-red*short-white*$n = 2nr(1-r)/4$.
- *E*(short-white) =short-white*short-white*$n = n(1-r)^2/4$.

Thus, we obtain the frequencies phenotype with *r*-values of stem length with flower color as following

- f_{k_1} : *f*(tall-red) $= (1-r^2)/4$.
- f_{k_2} : *f*(tall-pink) $= 2(1-r+r^2)/4$.
- f_{k_3} : *f*(tall-White) $= r(2-r)/4$.
- f_{k_4} : *f*(short-red) = $= r^2/4$.
- f_{k_5} : *f*(short-pink) $= 2r(1-r)/4$.

- f_{k_6} : f(short-white) $=(1-r)^2/4$.

Therefore, the probability of frequencies of appearance of observed phenotype number of steam length with flower color F_2 as above, in this case $m=6$.

5.2.3.3 The probability of linkage genotypes between stem color and flower color

Chapter 4 shows the *r*-value of linkage between stem color and flower color was estimated using only direct calculation methods. It confirmed that the reliability of the *r*-value, thus MLE methods were also performed.

Table 5.3 The recombination rate of genotypes values of stem color with flower color of F_2.

Gametes of F_2		**purple-red**	**purple-white**	**green-red**	**green-white**
		$\frac{1-r}{2}$	$\frac{r}{2}$	$\frac{r}{2}$	$\frac{1-r}{2}$
purple-red	$\frac{1-r}{2}$	$\left(\frac{1-r}{2}\right)^2$	$\frac{r(1-r)}{4}$	$\frac{r(1-r)}{4}$	$\left(\frac{1-r}{2}\right)^2$
purple-white	$\frac{r}{2}$	$\frac{r(1-r)}{4}$	$\left(\frac{r}{2}\right)^2$	$\left(\frac{r}{2}\right)^2$	$\frac{r(1-r)}{4}$
green-red	$\frac{r}{2}$	$\frac{r(1-r)}{4}$	$\left(\frac{r}{2}\right)^2$	$\left(\frac{r}{2}\right)^2$	$\frac{r(1-r)}{4}$
green-white	$\frac{1-r}{2}$	$\left(\frac{1-r}{2}\right)^2$	$\frac{r(1-r)}{4}$	$\frac{r(1-r)}{4}$	$\left(\frac{1-r}{2}\right)^2$

The expected genotype value of stem color with flower color as following

- E(purple-red) $= n(1-r)^2/4$.
- E(purple-pink) $= 2nr(1-r)/4$.
- E(purple-white) $= nr^2/4$.
- E(mixed-red) $= 2nr(1-r)/4$.
- E(mixed-pink) $= 2n(1-2r+r^2)/4$.
- E(mixed-white) $= 2nr(1-r)/4$.
- E(green-red) $= nr^2/4$
- E(green-pink) $= 2nr(1-r)/4$.
- E(green-white) $= n(1-r)^2/4$.

Here, we obtain the frequencies phenotype with recombination rate value of stem color with flower color as following

- f_{k_1} : f(purple-red)$=(1-r)^2/4$.

- f_{k_2} : f(purple-pink) $= 2r(1-r)/4$.
- f_{k_3} : f(purple-white) $= r^2/4$.
- f_{k_4} :f(mixed-red) $= 2r(1-r)$.
- f_{k_5} : f(mixed-pink) $= 2(1-2r+r^2)/4$.
- f_{k_6} : f(mixed-white) $= 2r(1-r)/4$.
- f_{k_7} : f(green-red) $= r^2/4$.
- f_{k_8} : f(green-pink) $= 2r(1-r)/4$.
- f_{k_9} : f(green-white) $= (1-r)^2/4$.

Therefore, The probability of frequencies of appearance of observed phenotype number of steam color with flower color F_2 as above, in this $m=9$.

5.3 Result and discussion

5.3.1 Linkage of stem length and stem color, and stem length and flower color characteristics

The Haibushi cultivar with tall-red flower crossed between Morocco cultivar with short-white flower. A and 2 of the F_2 population respectively were not segregated as the ratio of 3:6:3:1:2:1 into six different phenotypes, including tall-red, tall-pink, tall-white, short-red, short-pink, and short-white stem plants (Table 5.4). Thus, not so close, but the far linkage was estimated. The recombination value calculated was indicated that population 14-HAI and population 12-HAI were not detected recombinant value and showed independence, but others could be detected recombinant value between 0.39-0.46 (Table 5.5). In the confirmation of Maximum Likelihood methods to calculate the recombinant value, there was no recombination value in population-15-HAI; other population deviated from independence law and detected recombinant value between 0.44 and 0.46 (Table 5.5). However, the chi-square value of observed value and expected value detect significant differences; it was needed to check much more F_2 plants. Joint segregation of stem length and flower color also indicated that three of six populations did not detect recombinant value; in two of three populations detected recombination rate, the chi-square test, including *r*-value, detected significant differences between the estimated value and observed value, and the 8-HAI population was only detected $r=0.44$.

Because there was no significant gap of estimated recombination value between calculation methods by chi-square and Maximum Likelihood Estimation method, former methods were easier to understand than the latter for high school students.

Table 5.4 Joint segregation for stem length and stem color in F_2 population.

F_1 code	phenotypes	observed expected	F_2 phenotypes						*n*	chi-square *(1)*	degree of freedom	*p*-value
			tall purple	tall mixed	tall green	short purple	short mixed	short green				
14-HAI	stem length with stem color	observed	5	7	11	2	11	4	40	14.933	6	0.01065102
		expected	7.5	15	7.5	2.5	5	2.5				
	stem length with flower color	observed	6	8	9	4	10	3		9.867	6	0.07910255
		expected	7.5	15	7.5	2.5	5	2.5				
11-HAI	stem length with stem color	observed	7	11	5	2	11	5	41	11.423	6	0.04361304
		expected	7.68	15.37	7.6875	2.5625	5.125	2.5625				
	stem length with flower color	observed	5	14	4	1	13	4		16.691	6	0.00512456
		expected	7.68	15.37	7.6875	2.5625	5.125	2.5625				
12-HAI	stem length with stem color	observed	4	13	7	1	7	2	34	3.333	6	0.64874236
		expected	6.37	12.75	6.375	2.125	4.25	2.125				
	stem length with flower color	observed	2	15	7	2	6	2		4.196	6	0.52154478
		expected	6.37	12.75	6.375	2.125	4.25	2.125				
15-HAI	stem length with stem color	observed	6	21	12	2	9	7	57	6.719	6	0.24236733
		expected	10.68	21.37	10.6875	3.5625	7.125	3.5625				
	stem length with flower color	observed	8	21	10	5	8	5		1.994	6	0.84995362
		expected	10.68	21.37	10.6875	3.5625	7.125	3.5625				
8-HAI	stem length with stem color	observed	10	12	12	0	12	8	54	17.506	6	0.00363344
		expected	10.12	20.25	10.125	3.375	6.75	3.375				
	stem length with flower color	observed	12	10	12	4	9	7		10.642	6	0.05895926
		expected	10.12	20.25	10.125	3.375	6.75	3.375				
10-HAI	stem length with stem color	observed	6	6	5	6	6	8	37	25.414	6	0.00011587
		expected	6.93	13.87	6.9375	2.3125	4.625	2.3125				
	stem length with flower color	observed	5	11	1	6	7	7		22.82	6	0.00036542
		expected	6.93	13.87	6.9375	2.3125	4.625	2.3125				

(1); For 3:6:3:1:2:1

Table 5.5 Joint segregation for stem length and stem color including *r*-value in F_2 population.

F_1 code	observed expected	F_2 phenotypes						*n*	*r* (x^2)	x^2	degree of freedom	*p*-value	test confirmed
		tall purple	tall mixed	tall green	short purple	short mixed	short green		*r* (MLE)				
14-HAI	observed	5	7	11	2	11	4	40	0.5	14.93	5	0.01	recombinant
	expected	7.5	15	7.5	2.5	5	2.5		0.5				
11-HAI	observed	7	11	5	2	11	5	41	0.42	9.97	5	0.07	independent
	expected	8.44	15.50	6.80	1.80	4.99	3.44		0.44				
12-HAI	observed	4	13	7	1	7	2	34	0.5	3.33	5	0.64	independent
	expected	6.37	12.75	6.37	2.12	4.25	2.12		0.5				
15-HAI	observed	6	21	12	2	9	7	57	0.42	5.23	5	0.44	recombinant
	expected	11.73	21.55	9.45	2.51	6.94	4.79		0.5				
8-HAI	observed	10	12	12	0	12	8	54	0.39	13.88	5	0.016	recombinant
	expected	11.44	20.57	8.476	2.05	6.42	5.023		0.44				
10-HAI	observed	6	6	5	6	6	8	37	0.46	24.29	5	0.0001	recombinant
	expected	7.29	13.90	6.55	1.95	4.59	2.69		0.46				

Table 5.6 Joint Segregation for stem length and flower color including *r* value in F_2 population.

F_1 code	observed expected	F_2 phenotypes						*n*	*r* (x^2)	chi-square x^2	degree of freedom	*p*-value	test confirmed
		tall red	tall pink	tall white	short red	short pink	short white		*r* (MLE)				
14-HAI	observed	6	8	9	4	10	3	40	0.5	9.86	5	0.07	independent
	expected	7.5	15	7.5	2.5	5	2.5		0.5				
11-HAI	observed	5	14	4	1	13	4	41	0.44	15.92	5	0.007	recombinant
	expected	8.266	15.45	7.036	1.984	5.051	3.214		0.45				
12-HAI	observed	2	15	7	2	6	2	34	0.5	4.196	5	0.52	independent
	expected	6.375	12.75	6.375	2.125	4.25	2.125		0.5				
15-HAI	observed	8	21	10	5	8	5	57	0.5	1.9941	5	0.85	independent
	expected	10.69	21.38	10.69	3.563	7.125	3.563		0.5				
8-HAI	observed	12	10	12	4	9	7	54	0.44	9.55	5	0.088	independent
	expected	10.89	20.35	9.266	2.614	6.653	4.234		0.44				
10-HAI	observed	5	11	1	6	7	7	37	0.47	22.43	5	0.0004	recombinant
	expected	7.207	13.89	6.652	2.043	4.608	2.598		0.46				

5.3.2 The confirmation of recombination value by Maximum Likelihood Estimation linkage in the segregation between incomplete dominant pair, stem color, and flower color

In chapter 4, the couple of incomplete dominant characters were detected *r*-value by direct calculated methods. In this chapter, the Maximum Likelihood Estimate calculation was used to compare to chi-square methods, and differences between chi-square methods and Maximum Likelihood Estimation (MLE) methods were found in the case of far linkage. Thus, the confirmation of the linkage detection in chapter 4 by direct methods performed by MLE.

In a population of 10-HAI, whereas 8% differences were presented between chi-square methods and MLE, differences of other populations were less than 4.5%. These results suggested that detection of recombination value of nearly 0.5 as the nearly equal independent value was unstable at the small number of individuals in populations. So, it could not decide the recombination value obtained from chi-square methods could be used or not. However, understanding MLE methods to calculate recombinant value might be too tricky for Cambodian high school students. Nevertheless, the comparison of detection recombinant value between chi-square of direct calculated methods and MLE's should be calculated by the smaller recombinant value as 30%-40% of other coupled characters.

Table 5.7 Differences of *r* value between calculation using chi-square and calculation of MLE in stem color and flower color.

F_1 code	expected observed	purple red	purple pink	purple white	mixed red	mixed pink	mixed white	green red	green pink	green white	*n*	*r* (x^2) *r* (MLE)	chi-square	degree of freedom	p-value
14-HAI	expected	6.80	2.88	2.88	14.22	0.30	2.88	0.30	2.88	6.80	40	0.175	21.967	8	0.005
	observed	3	4	5	13	0	0	2	1	12		0.13			
11-HAI	expected	6.35	3.43	3.43	13.63	0.46	3.43	0.46	3.43	6.35	41	0.2125	14.494	8	0.0698
	observed	1	9	5	15	0	1	0	3	7		0.24			
12-HAI	expected	6.84	1.56	1.56	13.86	0.09	1.56	0.09	1.56	6.84	34	0.1029	5.636	8	0.6879
	observed	2	3	2	17	0	1	0	1	8		0.11			
15-HAI	expected	11.63	2.48	2.48	23.53	0.13	2.48	0.13	2.48	11.63	57	0.0965	12.364	8	0.1357
	observed	7	1	6	24	0	0	0	4	15		0.1			
8-HAI	expected	10.44	2.85	2.85	21.28	0.19	2.85	0.19	2.85	10.44	54	0.1204	14.031	8	0.081
	observed	9	1	7	15	0	2	0	3	17		0.13			
10-HAI	expected	5.68	3.13	3.13	12.22	0.43	3.13	0.43	3.13	5.68	37	0.2162	6.93	8	0.5442
	observed	7	5	3	8	0	1	1	5	7		0.13			

Chapter 6

The Applicability for Cambodian High School Biology Content

6.1 General discussion

In high school, the Cambodian biology curriculum was published by the Mistry of Education, Youth and Sport (MoEYS), 2011. The effectiveness of GAs (plant hormone chapter) was proposed in the grade 12th of content, while the Mendel genetic inheritance was in grade 11th (Table 6.1).

In this study, vine-less (dwarf) common bean was the excellent visible material because the whole shapes were significant differences between vine and vine-less. In Mendel's seven characters of a pea, dwarf characters also were used. It might be easy for the student to recognize a vine-less common bean instead of a dwarf pea because both species belonged to Leguminosae's family. This experiment can be used in my study of the exogenous GA and GA inhibitor application because the visible change of vine-less phenotype to normal phenotype and vine phenotype to vine-less phenotype was observed respectively. For education, while the same materials described in the textbook are better than other materials, it might be challenging to get seven mutant alleles of peas. So, it was considered to use materials instead of a pea. In this study, because some characters, flower color, stem color, vine-less, seed color, and pod shape in Japanese cultivars of common bean were found, these characters were considered to use genetic materials instead of seven characters pea. In contrast, Mendel reported that all seven characters inherited independently in Mendel's 3rd law, but in my research I found a genetic linkage between stem color and flower color. The possibility of genetic linkage was also found between stem length and stem color, and flower color in a cross combination of common bean. Although it was needed to pay attention to using common bean for Mendel's genetic materials, Morgan's description of genetic linkage found and clear linked between stem color and flower color could be used for the experimental class.

Although most textbooks described test cross progeny (doubled heterozygous characters multinomial double recessive character) of fruit fly, it was challenging to produce genetics linkage analysis through self-fertilized in plants. While it was correct that Mendel used pea and Morgan used fruit fly in the history of Genetics, it was hard for the teacher to prepare different materials by each content. It was considered that incomplete dominance character of stem length and flower color could be used for experimental class in Cambodia. In coupled complete dominant characters of F_2, it was impossible to use the high school experimental class, because it was impossible to estimate genotype from phenotype. Dominant phenotype included homozygous genotype, and heterozygous genotype consisted between a dominant allele and recessive allele. However, the incomplete dominant character was mostly estimated genotype from the phenotype of the F_2

population in chapter 5 described. For estimating genotype, it was considered essential to recognize the recombinant genotype for the student by meiosis. In this estimation, students could be considered that recombination occurred on a chromosome. In F_2 segregation, students learned Punnet square methods in independence. An independent case could be considered recombination value as 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 kinds of coupled genotype were described using *r* value. The formula of expected value could be 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 experimental genetic class included both Mendel's laws and genetic linkage, the independent color of stem and flower was considered better materials.

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 bean. Although these contents are divided into other grades, curriculum reconstructed could be needed as likely contents accumulating.

6.2 General conclusion

The experimental methods used to conduct experiments to study the effects of plant growth promotion GA and plant growth inhibitor B-NINE are good for using in biology education in Cambodia high school because these methods can be done by students. And on the other hand, the source of GA_3 and B-NINE inhibitor might be available at garden shops in Cambodia, and other equipment used can be purchased at markets. The genetic study using these common bean cultivars are also good for using in biology education in Cambodian high school because the crossing techniques and the cultivation methods can be done by students. In this part, the study of Mendel's laws of heredity which are generally studied in Cambodian biology textbook might be easy for students to understand. The extended study of Mendel's law and the gene linkage study and the calculation methods are very interesting, but they might be a little bit difficult for students.

6.3 Applicable ability for biology education in Cambodia

Experimental classes are preferred by students. However, the experiments used in my research are not appropriate for a teaching our experimental class in high school in Cambodia. Therefore, there are two ways to apply my research to improve biology education in high school in Cambodia. (1) The results of the experiments including figures, tables, and some calculation methods are good to

use to teach students in an hour teaching. For example, the figures showing the growth status of Morocco after receiving GA_3 treatment and of Haibushi after receiving B-NINE, and the tables of the effects of these plants growth hormones on stem and internode elongation can be used for students to inquire the effects plants hormone on plant growth. So the figures and tables from these experiments can be used to teach students in an inquiry-based learning (IBL) which is being used in high school in Cambodia. In Cambodia biology textbook, GA was described to promote plants growth, but there are not any experimental data to show students so they just image from the theory written in the textbook. (2) The experiments in my research takes long time, so they should be applied as research activities or in the Project-Based Learning (PBL). In these activities, students will be divided in to groups to design experiments, to do experiments, and to write their experimental research at the end of their experiment. In this research activity, students study based on their experiments, which is a good motivation for them to become researchers or scientists in the future. Table 6.1 showed the activities and the timeframe for students to conduct their research in Cambodian high school. It should be aware that Cambodia is a tropical country, so common bean (*Phaseolus vulgaris* L.) can be cultivated in the whole year.

Table 6.1 Currently of MoEYS's biology content and future improvement approach.

Grade	**title of lesson/chapter**	**content approach**	
		currently curriculum	**improvement approach**
11^{th}	Mendel's Genetic Inheritance	Semester 2	
	1) F_1 Progenies Producing		Semester 1
	2) F_2 Progenies Producing		Semester 2
12^{th}	Effectiveness of GA_3 on Plants	Semester 2	
	1) Effective of GA_3		Semester 1
	2) Effective of Inhibitor B.NINE		Semester 2

Acknowledgment

First of all, I would like to sincerely thank my supervisor, Professor **Dr. Juntaro KATO** of Aichi University of Education for his advice, guidance, motivation, and support my researches and my stay in Japan. I would like to sincerely thank **Professor Dr. Kiyoyuki OHSHIKA** and **Professor Dr. Mikio WATANABE** for their advices, comments, and correction when I was taking their courses, which contributed to my success in the study as well as the Master's research. I profoundly thank to His Excellency **Dr. Sovanna SIENG**, director of the National Institute of Education, for his support and motivation to me for pursuing my study at Aichi University of Education. And I profoundly thank to **Dr. Chansean MAM**, who always supports my research.

Finally, I would like to thank to Japanese government for providing me a full scholarship to study in Japan. I am thankful to the Aichi University of Education and the National Institute of Education in making a good cooperation for giving NIE's trainers a chance to study in the PhD and master course in Aichi University of Education in Japan.

Reference

Allard R. W., 1956. Formulas and Tables to Facilitate the Calculation Values in Heredity. Vol.24.

Binenbaum J., Weinstein R., and Shani E., 2018. Gibberellin Localization and Transport in Plants. Vol. 23(5).

Hatfield J.L, and Praeger J.H, 2015. Temperature extremes: Effect on plant growth and development. Volume 10, part A, p-4-10.

Hossain F., and Ara U., 2017. Effects of Gibberellin Acid (GA3) on Growth and Yield Parameters of French bean (Phaseolus Vulgaris L.). 43(1): 49-60.

Inglis J.R., and Robbins R.J., 2000. A History of Genetics.

Khafagi A.A.F., Ghamery A.A.E., Ghaly O.N., and Ragab O.G., 2018. Fruit and Seed Morphology of Some Species of Solanaceae. Vol. 38: 123-140.

Khan A.S., and Chaudhry N.Y., 2006. GA3 Improves Flower Yield in Some Cucurbits Treated with Lead and Mercury. Vol. 5(2), pp. 149-153.

Lanes B. A. Jaques L.B.A., Cavalho I.R, Szareski V.J, Pimentel J.R, Troyjack C., Dellagostin S.M, Mendonca M.T., Rosa T.C., Villela F.A., Souza V.Q., Aumonde T.Z., and Pedo T., 2019. Gibberellic Acid Utilization in Seed and Plants of Beans: Effect on Growth and Seeds Physiological Quality. Vol. 11, No.2, ISSN 1916-9752.

Laurentin H., and Benitez T., 2014. Inheritance of Seed Coat Color in Sesame. Vol. 49(4), pp. 290-295**).**

Lester D.R., Ross J.J., Davies P.J., and Reid J.B., 1997. Mendel's Stem Length Gene (*Le*) Encodes a Gibberellin *3ß-Hydroxylase*.

Liu X., and Hou X., 2018. Antagonistic Regulation of A.B.A. and G.A. in Metabolism and Signaling Pathways. Vol. 9(256).

Mam C., Yun K., Phen S., Noda Y., Funai H., Iwayama T., and Kato J., 2019. The Use of Dwarf Tomato Cultivars for Genetic Physiology Study applicable for School Education. Vol. 2(1), pp. 015-021. ISSN: 2169-3908.

McWilliam J.R, and A. W. Naylor A.W., 1967. Temperature and Plant Adaptation: Interaction of Temperature and Light in the Synthesis of Chlorophyll in Corn. 42,1711-1715.

Miceli A., Moncada A., Sabatino L., and Vetrano F., 2019. Effect od Gibberellic Acid on Growth, Yield, and Quality of Lave Lettuce and Rocket Grown in a Floating System.

Ngatia T.M., Shibairo S.I., Emongor V.E., and Obukosia S.D., 2004. Effect of Levels and Timing of Application of Gibberellic Acid on Growth and Yield Components of Common Beans. Vol. 12(2), pp.123-131.

Okuda M., 1964. Physiological Observation of the Gibberellin Effects on the Development and Growth of Plants. 81:1.

Omar Jabir B.M., Kinuthai K.B., Faroug M.A., Awad F.N., Muleke E.M., Amadzai Z., and Liu L., 2017. Effect of Gibberellin and Gibberellin Biosynthesis Inhibitor (Paclobutrazol) Applications on Radish (*Raphanus sativus*) Taproot Expansion and the Presence of Authentic Hormones. Vol. 19, No. 4, ISSN: 1814-9596.

Preacher K.J., 2020. Chi-Square Test Calculation software.

Rademacher W., 1991. Inhibitors of Gibberellin Biosynthesis: Applications in Agriculture and Horticulture.

Rosales R.J.G., and Galinato R.G., 2018. Effect of Gibberellic Acid on the Flowering and Yield Performance of Hybrid Variety of *Momordica charantia*. Vol. 5(6), I.S.S.S.N.: 2348-3997.

Rosolem C.A., Rodrigues J., 2003. Gibberellin and Cytokinin Effects on Soybean Growth. Vol. 60, n.3, p.537-541.

Schuch U.K., and Fuchigami L.H.,1990. Gibberellic Acid Causes Earlier Flowering and Synchronizes Fruit Ripening of Coffee. 9: 59-64.

Tanimoto E., Naohiko, and Masuda Y., 2006. Effect of Gibberellic Acid on Dwarf and Normal Pea Plants. Vil. 20, 291-298.

Temple S., Smitson J.B., Arregoces O.A.E., and Medina L.M.A.E., 1987. The Crossing of Beans.

Weaver R.F., and Hedrick P.W., 1992. Genetics. Second edition, pp. 107-112.

Yamaguchi S., Smith M.W., Brown R.G.S., Kamiya Y., and Sun T.P., 1998. Phytochrome Regulation and Differential Expression of Gibberellin 3 beta-Hydroxylase in Germinating Arabidopsis Seeds. Vol. 10, pp. 2115-2126.

Bateson W., 1901. Experiment in Plant Hybridization by Gregor Mendel (1865), English version.

Mendel J G., 1865. Experiment in Plant Hybridization

No Green Co., Ltd; https://.elife.jp.net/shopdetail.

https://item.rakuten.co.jp