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Research Article

The Use of Dwarf Tomato Cultivar for Genetic and Physiology Study Applicable for School Education

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The effect of Gibberellic acid (GA) on plant growth and Mendel's law of heredity were integrated in biology textbooks in many countries. However, there were not practical experiments described in those textbooks, especially in the Cambodian biology textbooks. Students had to learn based on the theories written in the books. Therefore, it was very difficult for them to understand the contents by imaging the terms from the books. In this study, we introduced experimental methods using dwarf tomato plant which is applicable for school education. The result of our research suggested that the causal factor of dwarf tomato using in this study was the less of GA and the inheritance of this dwarf followed Mendel's law of dominance and segregation. Dwarf tomato being used in this study was a good material and it will also be a good plant material for biological experiment in schools all over the world.

Keywords: Mendel's law, plant physiology, gibberellic acid, dwarf tomato, school education

INTRODUCTION

Plant physiology and genetic heredity were integrated in biology study in general education in many countries. Cambodian integrated the effect of plant hormones on plant growth and development in the 12th grade biology textbook, and Mendel's law of heredity in the 11th grade biology textbook (Yihoop *et al.*, 2009; Yihoop *et al.*, 2016), while Japanese integrated plant hormone in senior high school level (Akasaka *et al.*, 2014; Agata *et al.*, 2015) and Mendel's law of heredity in junior high school level (Arima *et al.*, 2016; Tsukada *et al.*, 2016). Gibberilic Acid (GA) is one of the major plant hormone groups studied in general education in Cambodia and Japan (Akasaka *et al.*, 2014; Agata *et al.*, 2015; Yihoop *et al.* 2009). GA was discovered by Japanese scientists (Kurosawa, 1926), and crystalized by Yabuta and Sumiki (1938). Currently, GA is commercially used to enhance growth and productivity of many crops and vegetables. GA_3 has been used to conduct experiments by various ways of application. Different concentration of GA_3 were applied on plants through different methods such as Miceli *et al.* (2019) applied in hydroponic solution for lettuce growth, Khan *et al.* (2006) sprayed on tomato, and Pal *et al.* (2016) sprayed on cucumber experiment. All parts of plants including height, branches, leaves per plant, leaf size, fresh weight, dry weight as well as flower and fruit, were measured in the study of the effect of GA_3 on plant growth and development (Khan *et al.*, 2006; Pal *et al.*, 2016; Miceli *et al.*, 2019). Khan *et al.* (2006) and Pal *et al.* (2016) reported that plants received GA_3 elongated longer than those in control. GA controls plant development by regulating plant physiological mechanism (Hooley, 1994). Lower GA level was well known for inducing dwarf phenotype. Semi-dwarf mutant of rice, IR-8, was known as green revolution rice and this dwarf phenotype was responded sensitively to exogenous GA. Ashikari *et al.* (2002) revealed that this

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dwarf phenotype was caused by mutation of GA biosynthesis enzyme, GA20ox-2. However, dwarf phenotype was also introduced by mutation associated Brassinosteroid (BR). The dwarf gene of Rice *d61* (Yamamuro *et al.*, 2000) and Barley *Uzu* (Chono *et al.*, 2003) were revealed as receptor mutation of BR. However, this mutant phenotype was known as pleiotropic effect expressed dwarf and wide leaf.

Mendel is a famous genetic scientist through his law of heredity, First law: Law of Dominance; Second law: Law of Segregation; and Third law: Law of Independent Assortment (Yihoop *et al.*, 2009; Gautam, 2018). There were many researchers conducted experiment to trace Mendel's law such Smykal *et al.* (2016) cited that in early 1900s de Vries, Correns, and Tschermak had published each research paper to confirm Mendel's second law (3:1). Even though there have been many papers published so far, there are not any paper that had yet been rejected Mendel's law of heredity.

The effect of GA on plant growth and Mendel's law of heredity were integrated in biology textbooks in many countries, but students might learn from the content in the textbooks without experimental practices. In our study, we introduced the experiments which are applicable for school education. Tomato is a good plant material for pupils to grow easily in kindergartens and to do simple research in primary schools in Japan. It is an important crop worldwide, and a model system for genetic studies in plant (Barone *et al.*, 2007). Yui *et al.* (2011) suggested that tomato is a good material to study genetics because many characters followed Mendel's law can be observed. In preliminary research, we purchased 25 cultivars of tomato seedlings sold in Japanese garden shops. All cultivars were transplanted to field for physiological growth examination. As a result, we found that one cultivar "Regina" was a dwarf tomato and other tomatoes were normal stem tomato plants. Among the normal type tomato plants, "Momo" showed the different stem height and fruit color. Therefore, we selected Regina and Momo for our study.

MATERIALS AND METHODS

Plant materials

In this research, self-fertilized plants of cultivar 'Regina' (dwarf type) and cultivar 'Momo' (normal type) were used as plant materials.

Physiology Study

Gibberellic acid (GA_3: Sigma-Aldrich) was dissolved in distilled water to make three different concentrations, 1mg/L, 10mg/L and 100mg/L. Seedlings with 3 true leaves of normal and dwarf types were used for exogenous GA_3 treatment in this study. Four seedlings were used in each concentration of GA_3 treatment including water treatment as control. Each seedling was sprayed on whole plant with 1.5mL of distilled water, 1mg/L, 10mg/L or 100mg/L GA_3 solution at 2 weeks after transplanting and the second treatment was done 2 weeks after the first treatment. The length of stem and internode were measured every week from the starting of GA_3 treatment by a ruler and caliper respectively.

Genetic Study

The dwarf type plant was crossed with the pollen of normal type plant. The crossing techniques were done as follows. From female plant (dwarf type): (1) selected young flower with green anthers at not full blooming stage (Figure 1a); (2) emasculation was done to pick up the anthers carefully by forceps without damaging of stigma (Figure 1b), and remained all parts of flower after removing anthers (Figure 1c). From male plant (normal type): selected a full-bloom flower and used another forceps without pollen to break down the anthers in order to make pollen grain attached to the forceps (Figure 1d). The forceps with the pollen grain was tapped on the stigma of female plant (Figure 1e). The flower pollinated were labelled (Figure 1f).

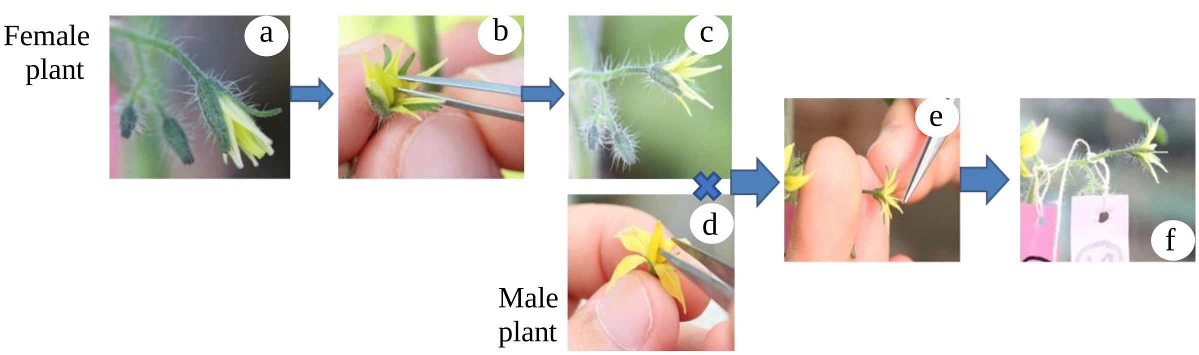

Figure 1: The method to cross tomato: a, young flower; b, emasculation; c, flower after removing anthers; d, breaking down the anthers; e, tapping pollen on stigma; and f, flower after crossing.

The successful of crossing could be confirmed by the youngest fruit observed in one week, and the mature fruits were obtained in 5 to 6 weeks from the crossing. The matured fruit was cut into small pieces, placed the pieces on a net, poured tap water to wash the pieces to remain the F_1 seeds on the net, and then the seeds were placed on Kimwipes paper for 3 days to dry. F_1 seeds were sown to check their phenotypes and some of F_1 plants were transplanted to field and kept for self-fertilization in order to produce F_2 seeds. F_2 seeds were sown in order to check their phenotypes. F_2 population were counted by pulling them out from the soil. The experiments were conducted for 2 times repeatedly by counting the seedlings within different stages.

Statically analysis

Statistical significances were analyzed by free software Real Statistics Using Excel (Charles Zaionts) with one factor ANOVA followed up option Turkey HSD with p-value < 0.05.

The statically test, probabilities of Chi-square was calculated by free online software for Calculation for the Chi-square test by Kristoper J. Preacher ©2010-2020.

RESULTS AND DISCUSSION

Physiology Study

Tomato plants responded to GA_3 treatment generally enhanced stem elongation comparing to control treatment in our experiments. Both dwarf type and normal type responded to 100mg/L GA_3 elongated the longest among other treatments, except normal type in week-2 (Figure 2). This elongating result indicated the same effect of GA on plant growth written in biology textbooks and previous researchers (Hooley, 1994; Khan *et al.*, 2006; Yihoop *et al.*, 2009; Akasaka *et al.*, 2014; Agata *et al.*, 2015; Pal *et al.*, 2016). Dwarf type tomato could elongate two times longer than the control treatment (Figure 2). This result suggested that GA_3 has strong effect on dwarf plant recovery.

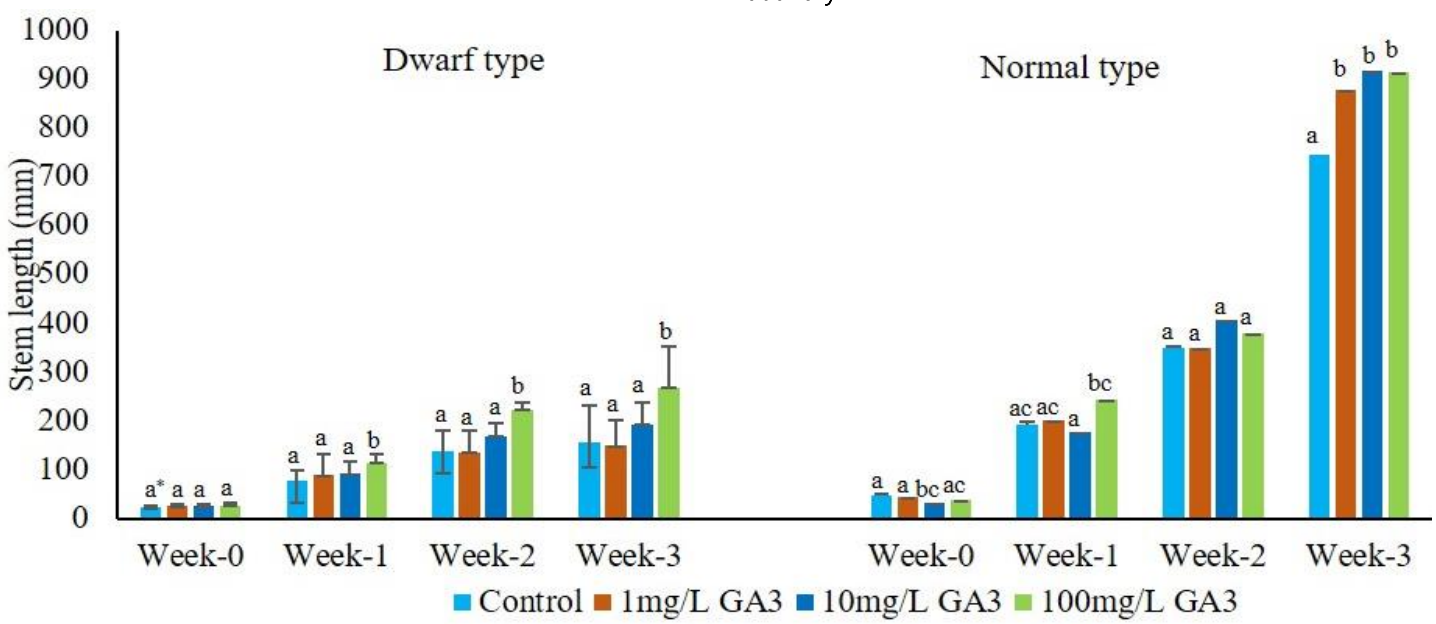

Figure 2: Average length of tomato plants 'Regina and Momo' under treatment of different GA_3 concentration. Week-0 is the started point of GA_3 treatment.
*Different letters on the bars show significant differences between treatments at a certain week by Real Statistics Using Excel (Charles Zaiontz) One Factor Anova follow-up option Turkey HSD p-value < 0.05

The effects of GA_3 on internode elongation were paralleled to those effects on stem growth. GA_3 had strong effects on internode elongation during the period of internode growing stage. The period of internode with potential growth was within one week after its induction. This growth was gradually decreased in the following weeks 'week-2' and some internodes were shorten in week-3 such as internode number 2 of dwarf type responded to 1.5mL of distilled water, 1mg/L GA_3, and 10mg/L GA_3 increased a lot at week-1, but this growth increased a little at week-2, and then they decreased a little at week-3 (Figure 3). The internode number 2 of normal tomato responded to 1.5 mL of distilled water or 100mg/L GA_3 were also a little shortened in week-3 (Figure 3). The shortening of internode indicated that stem or internode grows when it is young that cells have possibility to divide and the node is also enlarged during the time of internode elongation stop. GA_3 affected on young internode elongation, but this effect could not be observed in older internodes such as internode number 1 (Figure 3). The experiment on the effect of GA_3 on stem elongation did not explain only the effect of GA_3 on stem elongation, but also could explain students about growing stage of plant. Plant height increases depending on the growth of meristem at the top of plant, not depending on the older parts of the plants.

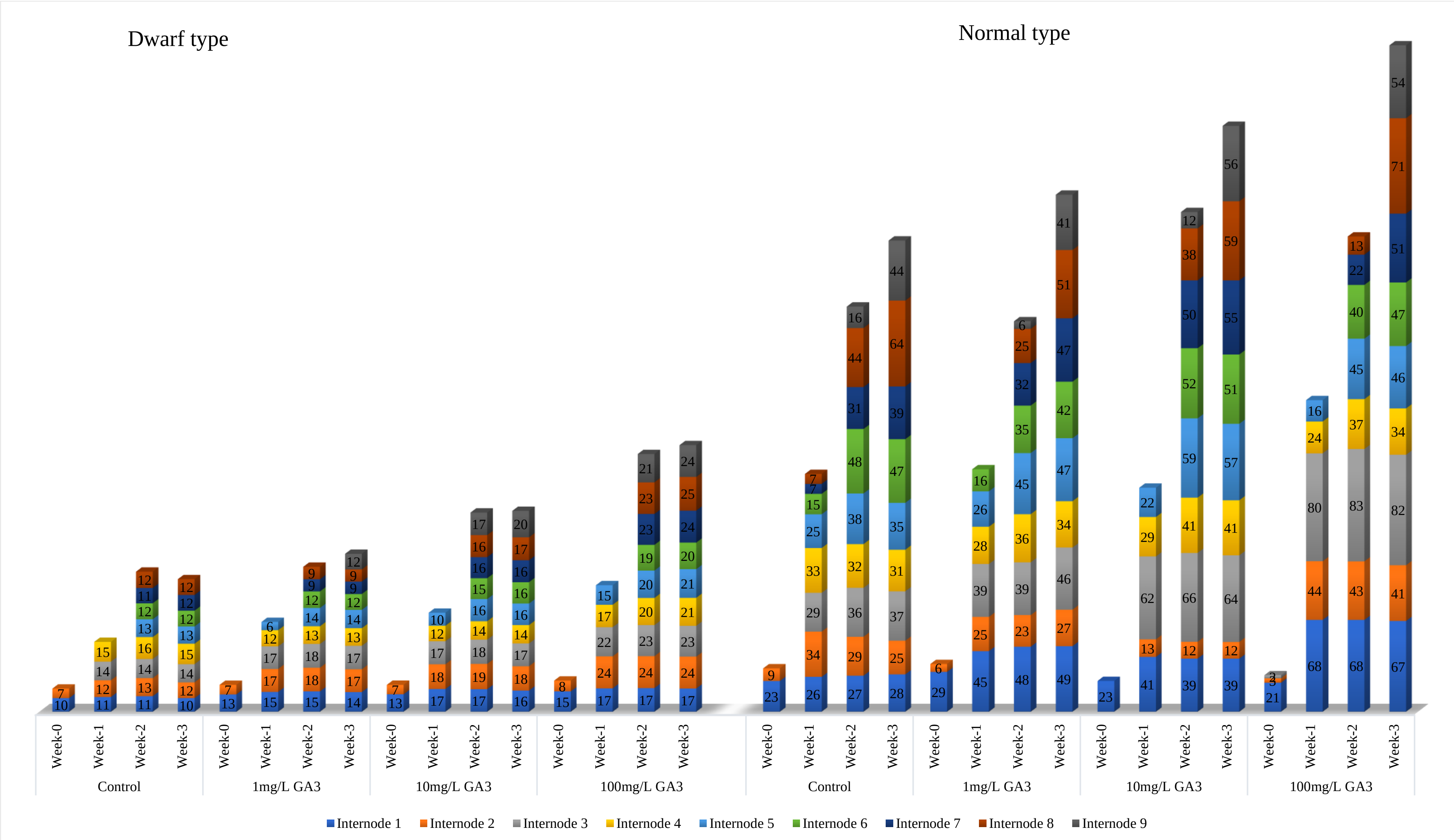

Figure 3: Average length of internode of tomato plants "dwarf and normal types" under treatment of different GA_3 concentration or distilled water from start point to 3 weeks later. The figure value in each internode image showed the average length (mm).

Genetic Study

F_1 plants resulted from the cross between dwarf type and normal type were all expressed the phenotype of normal type character in stem height. This result can confirm to Mendel's 1st Law of Dominance "the characters appear in F_1 generation are called dominant characters and the characters remain hidden are called recessive characters", "if the parents are homozygotes, all F_1 hybrids have the same phenotypes" and "tall is dominant on short" (Yihoop *et al.* 2009; Gautam, 2018).

There were segregations among F_2 population obtained from the cross between dwarf type and normal type, and normal-stem types were shown larger amount than dwarf ones at seedling stage (Figure 4). The F_2 segregation can be used to confirm Mendel's law of segregation, 3 dominant phenotypes and 1 recessive phenotype (3:1). F_2 population obtained from four F_1 plants in table 1 and six F_1 plants in table 2 showed high tendency to reach the ratio 3:1. Too young plants were more difficult to identify than the plants at 3 leaf stage or over. The F_2 populations obtained from six F_1 plants in table 1 mostly had only 1 true leaf that caused more difficult to identify between the normal stem and dwarf stem. The F_2 segregation of tomato plants having 3 true leaves were easier to identify between normal stem and dwarf stem, and had high probabilities to reach 3:1 ratio (Table 2). In table 2, even though F_2 segregation received from other four F_1 plants had low Chi-square p-value, 0.102 to 0.290, but this value also did not have statistic differences ($p>0.05$) to explain the F_2 segregation to follow Mendel' s law. Moreover, Mendel's experiment Chi-square is also low in terms of stem height, p-value: 0.436 (Table 3). In our experiment, F_2 segregation obtained from some F_1 plants were higher Chi-square value than those of Mendel's experiment with the highest p-value: 0.870 (Table 1, Table 2, and Table 3).

Figure 4: F_2 population resulted from the cross between Regina (short) and Momo (high), the photo was taken 3 weeks from seed sowing.

Table 1: F_2 segregation of normal stem and dwarf stem tomato at 1st true-leaf stage in F_2 population obtained from the cross between dwarf tomato 'Regina' and normal tomato 'Momo'

Parents of F_2 (F_1 Plant)	Number of F_2 Plants	F_2 Phenotypes (Number of plants)				Probabilities of Chi-square (p-value)
		Normal stems		Dwarf stems		
		Observed	Expected	Observed	Expected	
1	55	42	41.25	13	13.75	0.815
2	44	32	33.00	12	11.00	0.734
3	44	32	33.00	12	11.00	0.734
4	46	32	34.50	14	11.50	0.395
5	58	39	43.50	19	14.50	0.172
6	66	43	49.50	23	16.50	0.064
7	52	33	39.00	19	13.00	0.054
8	68	41	51.00	27	17.00	0.005
9	60	35	45.00	25	15.00	0.003
10	57	31	42.75	26	14.25	0.0003

Table 2: F_2 segregation of normal stem and dwarf stem tomato at 3rd true-leaf stage in F_2 population obtained from the cross between dwarf tomato 'Regina' and normal tomato 'Momo'

Parents of F_2 (F_1 Plant)	Number of F_2 Plants	F_2 Phenotypes (Number of plants)				Probabilities of Chi-square (p-value)
		Normal stems		Dwarf stems		
		Observed	Expected	Observed	Expected	
1	50	37	37.50	13	12.50	0.870
2	63	48	47.25	15	15.75	0.827
3	72	52	54.00	20	18.00	0.586
4	65	46	48.75	19	16.25	0.431
5	69	55	51.75	14	17.25	0.366
6	74	59	55.50	15	18.50	0.347
7	67	54	50.25	13	16.75	0.290
8	66	54	49.50	12	16.50	0.201
9	73	60	54.75	13	18.25	0.156
10	72	60	54.00	12	18.00	0.102

Table 3: F_2 segregation of Mendel's experiments on garden peas

Characteristics	F_2 Phenotypes				Probabilities of Chi-square (p-value)
	Observed	Expected	Observed	Expected	
Stem height	Tall stems		Short stems		0.436
	787	798	277	266	
Flower color	Purple flowers		White flowers		0.544
	705	697	224	232	
Seed color	Yellow seeds		Green seeds		0.897
	6022	6017	2001	2006	
Seed character	Round seeds		Wrinkled seeds		0.608
	5475	5494	1850	1831	
Pod color	Green pods		Yellow pods		0.502
	428	435	152	145	

Source of data: Cambodian biology textbook (Yihoop *et al.*, 2009) and Genetics Second Edition (Weaver R.F. and Hedrick P.W., 1992)

POSSIBILITY SCHOOL EDUCATION APPLICATION METHODS

The experiments on the effects of GA_3 on tomato physical growth and the use of tomato to trace Mendel's law needs a few months to finish so these experiments should be applied as extra-research activities for students at school. These experiments are applicable for school education because students are familiar with tomato, GA_3 source could be purchased at a chemical store or online shop, and the crossing methods are also easy for students to handle. The implication of the application is that students need long time to take care of their tomato plants. The method of spraying GA_3 on stem is a good technique to apply GA_3 because the students in developing countries including Cambodia are not familiar with hydroponic growing. Even though experiment practice is preferable, the use of these experimental results in classroom teaching is another method to apply the use of tomato to teach physical growth and genetic study. The data in these experiments are evidence to help students understand the effect of GA_3 on plant growth and to confirm Mendel's law of heredity. While Chi square test is too difficult to high school students, teachers can know students counted data match expected value. When p-value is small but bigger than 0.05, students may misunderstand their observed data does not follow Mendel's Law. In that case, we propose teachers to ask their students to compare between observed value and expected value by themselves. They will understand that the observed value is not so different from expected value.

CONCLUSION

Dwarf tomato 'Regina' is a good plant material for physiology and genetic study which are applicable for school education. Normal stem tomatoes are observed in many farms, but dwarf tomato could not be observed a lot at a farm or daily life. Therefore, the selection of tomato plants including dwarf and normal plants are essential for the study. The theories describing the effect of GA on plant growth and Mendel's law of dominance and segregation could be confirmed by the experiments using tomato plants and they are applicable for school education because the experiments can be conducted by using simple equipment that students can find in their daily-life equipment.

REFERENCES

Ashikari M, Sasaki A, Ueguchi-Tanaka M, Itoh H, Nishimura A, Datta S, Ishiyama K, Saito T, Kobayashi M, Khush G S, Kitano M, Matsuoka M. (2002). Loss-of-function of a rice gibberellin biosynthetic gene, GA20 oxidase (GA200x-2), Led to the Rice 'Green Revolution'. Breeding Science 52 : 143-150.

Barone A, Chiusano M L, Erocolano M R, Giuliano G, Grandillo S, Frusciante L. (2007). Structural and functional genomics of tomato. International Journal of Plant Genomics. 2008: 1-12.

Chono M, Honda I, Zeniya H, Yoneyama K, Saisho D, Takeda K, Takatsuto S, Hoshino T, Watanabe Y. (2003). A semidwarf phenotype of barley Uzu results from a nucleotide substitution in the gene encoding a putative brassinosteroid receptor. Plant Physiology. 133: 1209–1219.

Gautam A. (2018). Mendel's laws. Springer International Publishing AG, part of Springer Nature. 2018:1-3.

Hooley R. (1994). Gibberellins: perception, transduction and responses. Plant Molecular Biology. 26: 1529-1555.

Khan M M A, Gautum C, Mohammad F, Siddiqui M H, Naeem M, Khan M N. (2006). Effect of Gibberellic acid spray on performance of tomato. Turk J Biol. 30:11- 16.

Kurosawa E. (1926). Experimental studies on the nature of the substance secreted by the "bakanae" fungus. Nat Hist Soc Formosa 16: 213–227.

Miceli A, Moncada A, Sabatino L, Vetrano F. (2019). Effect of gibberellic acid on growth, yield, and quality of leaf lettuce and rocket grown in floating system. Agronomy. 9: 1-22.

Pal P, Yadav K, Kumar K, Singh N. (2016). Effect of gibberellic acid and potassium foliar sprays on productivity and physiological and biochemical parameters of parthenocarpic cucumber cv. 'seven star F_1'. Journal of Horticultural Research. 24 (1): 93 -100.

Smykal P, Varshney R K, Singh V K, Coyne C J, Domoney C, Kejnovsky E, Warkentin T. (2016). From Mendel's discovery on pea to today's plant genetics and breeding. Theor Appl Genet. 129: 2267-2280.

Weaver R F, Hedrick P W. (1992). Fundamental of Mendelian genetics. Genetics. Second edition: 15-23.

Yabuta, T.; Sumiki, Y. (1938). On the crystal of gibberellin, a substance to promote plant growth. J. Agric. Chem. Soc. Japan 14: 1526.

Yamamuro C, Ihara Y, Wu X, Noguchi T, Fujioka S, Takatsuto S, Ashikari M, Kitano H, Matsuoka M. (2000). Loss of function of a rice brassinosteroid insensitive1 homolog prevents internode elongation and bending of the lamina joint. The Plant Cell. 12: 1591–1605.

Yui S, Kataoka S, Honjo M. (2011). Comparison of Cultivated plants for the Basic Experiment of Genetics — Tomato as a Teaching Material— Jpn. J. Biol. Educ. 51 (1・2): 9-20.

Cambodian Biology Textbooks (in Khmer)

Yihoop Y, Vanny H, Somaly S, Soa V. (2009). 11th grade biology textbook. Publishing and Distributing House.: 74-77.

Yihoop Y, Vanny H, Somaly S, Soa V. (2016). 12th grade biology textbook. Publishing and Distributing House.: 43-44.

Japanese Biology Textbooks (in Japanese)

Agata K, Fujimoto H, Furumoto H, *et al.* (2015). Biology (Certified by MEXT, No. 304) for senior high school. Daiichi Gakushu-sha Co. Ltd.: 255-259.

Akasaka K, Hirata K, Iijima K, *et al.* (2014). Biology (Certified by MEXT, No. 302) for senior high school. Shinko Shuppan-sha Keirin-kan Co. Ltd. pp.: 297-300.

Arima A, Kobayashi M, Aoki K, *et al.* (2016). World of science Part 3 for junior high school. Dainippon Tosho: 94-97.

Tsukada M, Ohya Y, Eguchi T, *et al.* (2016). Gateway to the Future, Science, Part 3. Shinko Shuppan-sha Keirin-kan: 14-19.

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