

The Effect of Self and Cross Pollination Using Honey Bee and Development in Complete Fruit and Seed Production in the Self-Incompatible Angiosperm in *Brassica Rapa*

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Submitted: 2023, June 01; Accepted: 2023, June 29; Published: 2023, June 30

Citation: Moayeri, M. (2023). The Effect of Self and Cross Pollination Using Honey Bee and Development in Complete Fruit and Seed Production in the Self-Incompatible Angiosperm in *Brassica Rapa*. *OA J Applied Sci Technol*, 1(1), 37-45.

Abstract

Pollination was a process of transferring pollen grains from the male anther of a flower to the female stigma for plant reproduction and was an important mechanism in the life cycle of plants. This experiment involved a life cycle of *Brassica rapa* which was a member of the mustard or cabbage family. *Brassica rapa* was exceptional in its rapid development from seed to seed within four weeks. Morphological aspects and characterization of pollination was an important topic for developing fruit and seed production in the life cycle of the plant. The objective of this experiment was to assess the effects of cross-pollination on fruit and seed production of *Brassica rapa*. The major methods for this experiment required to use two Styrofoam quads as a control and a cross-pollinated watered the two seeds in each cell for three days of experiment and used honey bee for cross-pollination. The average of developed fruit in cross-pollination was 3.791 and the average of developed fruit in self-pollination was 1.170. The average of the mean seed in cross-pollination was 4.307 and the average the mean seed in self-pollination was 1.198. Therefore, the rate of developed fruit and the mean number of seeds were higher on cross-pollination than on self-pollination. Conversely, the average of undeveloped fruit in cross-pollination was 9.677 and the average of undeveloped fruit in self-pollination was 13.937. This result indicates that the rate of undeveloped fruit was higher on self-pollination than on cross-pollination. The means of the two groups (self-pollinated and cross-pollinated) were significantly different because the P-value for developed fruits, the mean number of seeds, and undeveloped fruits were 0.000, 0.000, and 0.001 respectively. This study concluded that regulated hydration of pollen for pollination and balanced hydration and dehydration cycle to pollen grains are the key result of this experiment.

Keywords: *Brassica Rapa*, Cross Pollination, Fruit and Seed Production, Germination, Honey Bee

1. Introduction

This paper explained about the plantation of *Brassica rapa* seeds to study the growth rate and distinguish between undeveloped ovaries and ovaries that matured into fruit with viable seeds. Pollination is an important process in the life cycle of *Brassica rapa*. The absence of cross-pollination decreased the seed yields and developed seed in the subsequent generation of *Brassica rapa*. The plant experimental project used *Brassica rapa* for its rapid development as a model organism in plant experimental project. *Brassica rapa* was a member of the mustard or cabbage family [1]. The characteristics of *Brassica rapa* were herbaceous, self-incompatible for pollination, and lack of seed dormancy with rapid seed maturation. The rapid growth of *Brassica rapa* occurred at room temperature with petite growth habit under continuous illumination. *Brassica rapa* adapted to different environments across a very wide geographic area [1]. *Brassica rapa* has many small leaves, branches, and bright yellow flowers. *Brassica rapa* needed cross-pollination to produce seed and embryo development and it required mechanical pollen transfer [1]. Pollen grains released from anthers and captured

on the surface of the stigma to obtain resources and water for germination and pollen tube elongation. Pollen tube penetrated the stigmatic cell wall and it grew into the ovary for fertilization [2]. Additionally, two sperm cells released from the pollen tube to fertilize the egg cells, resulting in seed development [3].

Insects such as honey bee played an important role in pollen vectors and pollinated almost all flowering plant species. Honey bee mediated pollination and stigmas needed to select suitable pollen for successful fertilization. Self-incompatibility associated with pollen selectivity and recognized as one of the methods to prevent self-fertilization [4]. Self-incompatibility was a mechanism in flowering plants that prevented inbreeding and enabled plants to reject their pollen to prevent self-fertilization and promote outcrossing [1]. Self-incompatible plants such as *Brassica rapa* required pollen transfer from plant to plant. Therefore, honey bee pollination increased the seed yield and larger grain [5]. The objective of this study was to assess the effects of cross-pollination on fruit and seed production of *Brassica rapa*. Therefore, this experimental study

focused on pollen behaviors during pollination and analyzed pollen behaviors during cross-pollination in *Brassica rapa*. This approach demonstrated that pollen exhibits different behaviors on an individual stigma in cross-pollination and the ratios of the different types of pollen behaviors such as undeveloped fruit, developed fruit, and seeds were critical for successful pollination.

2. Methods

We conducted the study in a laboratory setting located at the University of Victoria (UVic) from January 2020 to March 2020 (6). We used one Styrofoam quad as control and one Styrofoam quad as cross-pollinated and inserted one wick into each cell of each quad and extends them 1 cm through the bottom of the quad. The experiment required to moisten the potting soil until damp and fill each cell halfway to the top with adding 2 or 3 drops of fertilizer pellets and top up the cell with soil. We planted two seeds in each cell at a depth of 4 mm and watered them until water drips from the wick for three days of experiment and placed the quads on the water mat. As we watered the quads and cotyledons extended above the soil, we snipped the plant at ground level to one plant per cell in the first week of growth after about a week into the experiment. We also used a honey bee thorax to cross-pollinate the plants as soon as each flower opened for three consecutive days and rubbed the thorax on the mature anthers of open flowers in one plant, and touched the thorax to the stigma of open flowers in another plant. We repeated this process only on a cross-pollinated quad over several days on as many flowers as possible. We collected our data on the number of developed fruits, the number of undeveloped fruits, and the mean number of seeds/fruits. There were 40 number of replications for this experiment using Microsoft Office Excel 2010 and Minitab 16. We used Microsoft Office Excel 2010 to plot the averages and standard deviation along with formal graphs and standard deviation bars and Minitab 16 to perform a Welch's approximate t-test to find the P-value. The table below demonstrated the summary statistics for this (control, treatment) experiment include average, standard deviation and P-value for the number of developed fruits, number of undeveloped fruits, and the mean number of seeds/fruits.

3. Results

Pollen exhibited various behaviours in self and cross-pollination in three observed different categories of undeveloped fruit,

developed fruit, and seed production in this experiment. Hydration expanded pollen grains at different speed rates on each stigma, but some pollen grains did not hydrate or responded slowly within the period of observation. Additionally, pollen grains exhibited different morphological behaviours on each stigma in both self and cross-pollination. The rate of developed fruit and the mean number of seeds were higher on cross-pollination than on self-pollination. Thus, the rate of pollen expansion and germination were higher in cross-pollination than on self-pollination (Figure 2 and Figure 3). Conversely, the rate of undeveloped fruit and pollen without morphological changes were higher on self-pollination than on cross-pollination (Figure 1).

We synthesized the raw data and evaluated the degree of pollen tube penetration into the stigma and production of seeds by test pollination and calculated the average and standard deviation. The average of developed fruit in cross-pollination was 3.791 ± 7.995 and the average of developed fruit in self-pollination was 1.170 ± 1.897 (Table 1). Additionally, the average of the mean seed in cross-pollination was 4.307 ± 4.119 and the average the mean seed in self-pollination was 1.198 ± 2.060 (Table 1). Therefore, the rate of developed fruit and the mean number of seeds pollen was higher on cross-pollination than on self-pollination (Table 1). Conversely, the average of undeveloped fruit in cross-pollination was 9.677 ± 10.364 and the average of undeveloped fruit in self-pollination was 13.937 ± 12.063 (Table 1). This result indicates that the rate of undeveloped fruit was higher on self-pollination than on cross-pollination. The raw data demonstrated numerous specific examples within the trends (Table 3). For example, plant cell two in group one showed that there was only 1 developed fruit and 8 seeds in cross-pollination and there was not any developed fruit and seed in self-pollination (Table 3). Additionally, there were 9 undeveloped fruit in cross-pollination and 11 undeveloped fruit in self-pollination (Table 3).

The P-value for both the developed fruit and the mean number of seeds were 0.000 and the P-value for undeveloped fruit was 0.001. Therefore, based on the evidence that we have, we rejected the null hypothesis and concluded that the means of the two groups (self-pollinated and cross-pollinated) were significantly different because the P-value was less than 0.05 (Table 2).

	Number of developed fruit (control)	Number of developed fruit (treatment)	Mean number of seed (control)	Mean number of seed (treatment)	Number of undeveloped fruit (control)	Number of undeveloped fruit (treatment)
Average	1.170	3.791	1.198	4.307	13.937	9.677
Standard Deviation	1.897	7.995	2.060	4.119	12.063	10.364

Table 1: The average and standard deviation of the two groups (control and treatment) for developed fruits, mean number of seeds, and undeveloped fruits implied that the rate of developed fruit and the mean number of seeds was higher on cross pollination than on self-pollination and the rate of undeveloped fruit was higher on self-pollination than on cross pollination.

	Developed fruit (control and treatment)	Mean number of seed (control and treatment)	Undeveloped fruit (control and treatment)
P-Value	0.000<0.05	0.000<0.05	0.001<0.05

Table 2: The means of the two groups (self-pollinated and cross pollinated) are significantly different because the P-value for developed fruits, mean number of seeds, and undeveloped fruits were 0.000, 0.000, and 0.001 respectively. Therefore, we can reject the null hypothesis in all three groups because the P-value is less than 0.05.

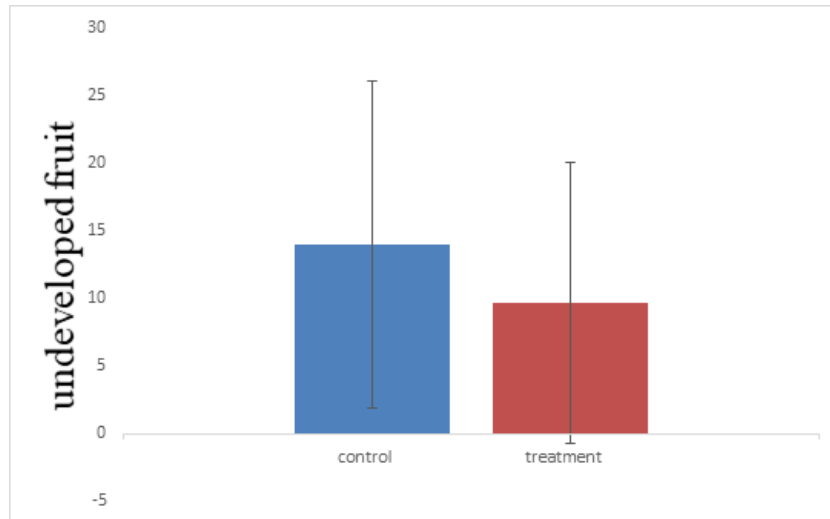


Figure 1: The average of undeveloped fruit in cross pollination was 9.677 and the average of undeveloped fruit in self-pollination was 13.937 and the error bars illustrated the standard deviation. This result indicates that the rate of undeveloped fruit was higher on self-pollination (control) than on cross pollination (treatment).

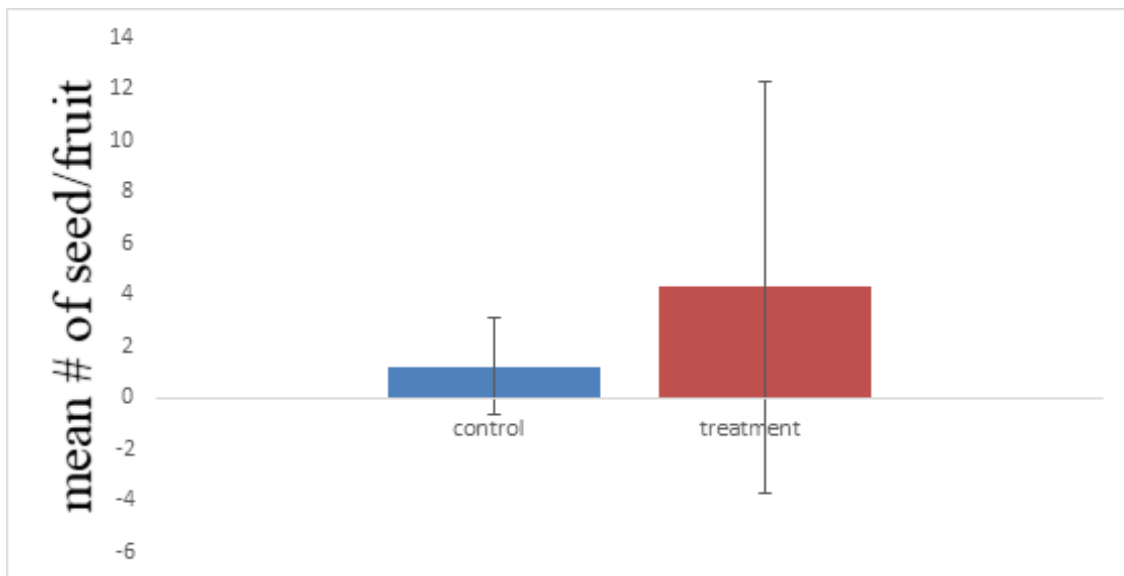


Figure 2: The average of the mean seed in cross pollination was 4.307 and the average the mean seed in self-pollination was 1.198 and the error bars illustrated the standard deviation. This result indicates, the mean number of seeds was higher on cross pollination (treatment) than on self-pollination (control).

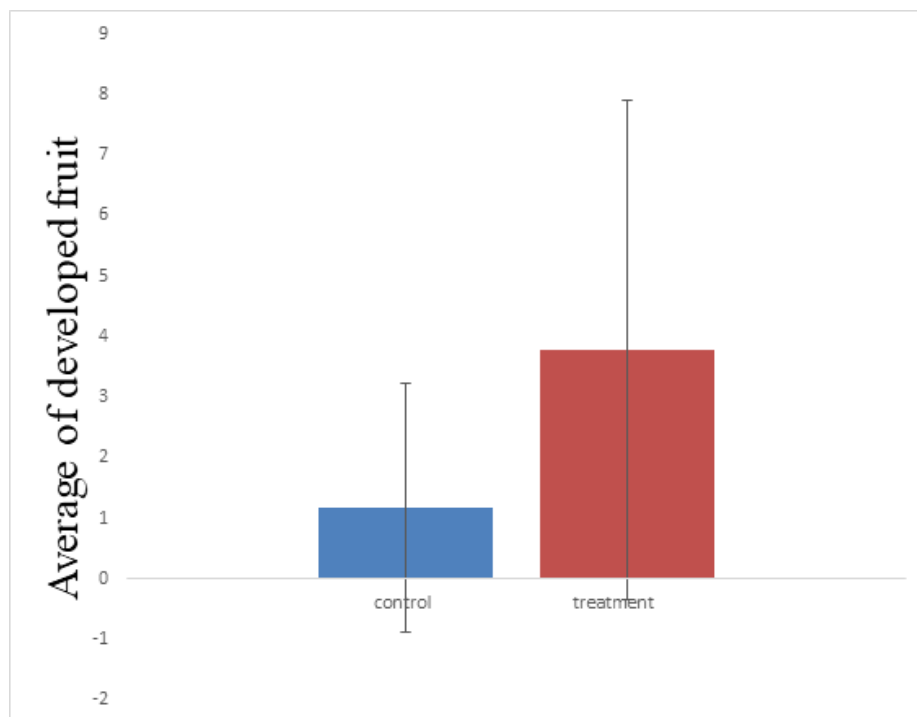


Figure 3: The average of developed fruit in cross pollination was 3.791 and the average of developed fruit in self-pollination was 1.170 and the error bars illustrated the standard deviation. This result indicates that the rate of developed fruit was higher on cross pollination (treatment) than on self-pollination (control).

4. Discussion

The objective of this experiment was to assess the effects of cross-pollination on fruit and seed production of *Brassica rapa*. This study also identified the specific types of pollen behaviour on individual stigmas in both self and cross-pollination in *Brassica rapa*. The major results of this study demonstrated that pollen grains exhibited different morphological behaviours on each stigma in both self and cross-pollination. Moreover, the rate of developed fruit and the mean number of seeds pollen were higher on cross-pollination than on self-pollination and the rate of undeveloped fruit and pollen without morphological changes were higher on self-pollination than on cross-pollination.

Contraction and pulsation were two major notable characteristics of pollen behaviours. Pollen grains shrunk shortly after successful hydration in contraction and repeated expansion and contraction caused pulsation [1]. Another relevant research on this field suggested that rapid dehydration in pulsation has an actively regulated dehydration system in pollination. This dehydration system regulated against the hydration system in pollination which was critical for water transport from the papilla to pollen [7]

Germination was also another types of pollen behaviour that cross-pollinated grains that hydrated sufficient enough and pollen tubes elongated into the stigma. In contrast, self-pollen tubes did not penetrate into the stigma [1]. Pollen tubes penetrated into the stigma after successful hydration of cross-pollinated grains through the stigmatic cuticle [8].

The self-incompatibility mechanism on pollination in *Brassica*

rapa depended on the percentage and speed of pollen hydration during pollination [9]. Despite in self-pollination, the viability level for reproduction in most pollen samples were sufficient in cross-pollination. Thus, self-pollination exhibited lower and cross-pollination exhibited a higher percentage of hydration which is consistent with self-incompatibility. This result indicated that germination has a higher percentage in cross-pollination, and contraction has a higher percentage of self-pollination. Therefore, self-pollination loses the required water for successful germination after hydration [10]. Another key result of this study suggests that there was a competitive and collaborative response in dehydration and hydration on self-pollination. This mechanism also implied that although the speed and percentage of hydration in both self and cross-pollination were the same, there was a higher percentage of pulsation on self-pollination and there was the balance between hydration and dehydration of pollen grains on pollination [8].

5. Conclusion

There were some seeds and fruit that did not fully develop or germinate successfully for this experiment, due to the time constraints. Additionally, we had to cross-pollinate the plants at different times during the experiment because some plants grew faster or slower than the others, however, we cross-pollinate all the plants in one laboratory setting. Also, the amount of collected data was too small to make a reliable analysis which all can be the source of errors for this experiment. As the above factors may have influenced the results, repeating the experiments with larger sample size and provide enough time for plants to develop fruits and seeds will significantly yield more data and allow the experiments to reach a solid conclusion. For future research,

diversity and comparison of pollinator insects for cross-pollination other than a honey bee (e.g. butterflies or wasps) in relation to seed and fruit development of *Brassica rapa* will be a solid topic as pollinators provide the key of the ecosystem to almost all flowering plants species. Performing self and cross-pollination with the emasculated flower buds and pollen from freshly opened flowers under room temperature will be another topic for future research [1]. Additionally, studying the specific aspects of honey bee behavior and their impact on *Brassica rapa* pollination efficiency. This could involve investigating factors such as bee visitation rates, foraging behavior, pollen transfer, and pollen viability. Moreover, pollinator abundance and diversity. This could involve investigating the influence of honey bee abundance and diversity on *Brassica rapa* pollination. This research could explore the potential benefits of maintaining diverse pollinator communities, including both managed honey bees and wild pollinators, to enhance crop productivity [11].

Based on the observations from pollen behaviours in pollination, we can conclude that water supply and pollinator were the main important steps in successful pollination. This stage consisted of hydration, rehydration and dehydration steps that involved water transport to pollen grains. This study also provided insight into the importance of pollinators such as honey bee for plant cross-pollination which played a significant role in the development of fruit and seed in *Brassica rapa*.

In conclusion, the effect of cross-pollination using honey bees in self-incompatible angiosperms like *Brassica rapa* is highly beneficial for fruit and seed production. Honey bees contribute to overcoming self-incompatibility barriers, improving pollination efficiency, increasing genetic diversity, and ultimately enhancing the yield and quality of the crop. Future research in this field can further explore the specific mechanisms underlying these effects, investigate optimal pollination management practices, and examine the potential impacts of changing environmental conditions on honey bee-mediated cross-pollination in *Brassica rapa*. Such research endeavors will provide valuable insights for optimizing agricultural practices and ensuring sustainable production of this important crop.

Declaration of Interests

The author, Mahbod Moayeri certify that there is NO affiliations with or involvement in any organization, or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript. The author also declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Data Availability Statement

The author confirms that the data supporting the findings of this study are available within the article in its supplementary (appendix part) materials.

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Appendix

GROUP #	CELL/ PLANT	# of developed fruit		mean # of seed/fruit		# undeveloped fruit	
		Control	Treatment	Control	Treatment	Control	Treatment
1	1	4	4	3.75	3.5	8	16
1	2	0	1	0	8	11	9
1	3	1	0	12	0	8	12
1	4	0	6	0	5	0	13
2	1	1	3	0	6.666666667	26	0
2	2	0	3	0	1	19	4
2	3	3	NA	0.67	NA	20	NA
2	4	0	NA	0	NA	8	NA
3	1	0	2	0	5.5	7	12
3	2	0	3	0	3.333333333	22	23
3	3	0	3	0	1.666666667	21	3
3	4	NA	5	NA	6.4	NA	2
4	1	0	71	0	14.2	3	0
4	2	0	62	0	12.4	5	0
4	3	0	15	0	2.8	9	5
4	4	2	30	1	5	24	0
5	1	0	3	0	9	33	4
5	2	0	1	0	13	30	8
5	3	0	6	0	5.8	26	22
5	4	0	2	0	1	23	43
6	1	2	0	1.5	0	30	20
6	2	5	3	1.8	1	15	23
6	3	7	0	4	0	10	47
6	4	1	3	1	3.3	54	16
7	1	0	2	0	4	4	4
7	2	1	0	2	0	17	5
7	3	1	2	2	2.5	7	31
7	4	0	0	0	0	10	25
8	1	0	1	0	9	2	6
8	2	0	10	0	1.6	13	8
8	3	2	9	5.5	3.7	5	0
8	4	0	2	0	7	29	26
9	1	2	3	2	11.3	17	2
9	2	0	6	0	8.83	8	7
9	3	2	5	6.5	2.8	17	19
9	4	0	5	0	2.2	15	4
10	1	0	4	0	3.75	24	2
10	2	0	1	0	4	6	3
10	3	0	4	0	2	23	7
10	4	1	4	0.5	7.75	6	0
11	1	3	0	4.33	0	3	7
11	2	0	0	0	0	1	0
11	3	1	0	1	1	16	1
11	4	0	0	0	0	4	0
12	1	0	0	0	0	31	11

12	2	2	0	5	0	14	1
12	3	0	0	0	0	0	0
12	4	5	0	4.8	0	4	0
13	1	1	0	7	0	15	0
13	2	0	0	0	0	3	3
13	3	0	0	0	0	3	20
13	4	0	1	0	4	12	13
14	1	2	12	1	4.5	11	10
14	2	2	1	1	6	12	2
14	3	0	1	0	1	6	3
14	4	1	4	1	2.25	32	9
15	1	0	4	0	8.25	11	1
15	2	1	0	1	0	7	9
15	3	0	0	0	0	13	1
15	4	0	0	0	0	7	8
16	1	1	2	2	1.5	2	4
16	2	0	0	0	0	6	7
16	3	0	1	0	1	1	13
16	4	0	1	0	1	2	13
17	1	0	5	0	6.8	10	2
17	2	9	0	2.89	0	0	0
17	3	0	0	0	0	7	3
17	4	3	0	3	0	4	9
18	1	5	1	3	2	1	9
18	2	0	0	0	0	3	0
18	3	0	2	0	4	0	9
18	4	1	0	3	0	2	5
19	1	0	7	0	5.142857143	0	9
19	2	0	0	0	0	0	8
19	3	0	0	0	0	0	6
19	4	0	0	0	0	0	9
20	1	0	1	0	4	3	4
20	2	0	0	0	0	2	5
20	3	0	0	0	0	0	5
20	4	0	0	0	0	1	3
21	1	1	3	2	7.666666667	20	5
21	2	0	6	0	5.333333333	22	9
21	3	1	2	1	5	20	32
21	4	1	2	1	6	28	39
22	1	1	2	3	7	24	25
22	2	2	3	1	2.3	32	34
22	3	2	5	1	3.4	35	6
22	4	1	7	1	6.4	36	2
23	1	2	5	2	2.4	10	3
23	2	3	4	1	1.75	20	4
23	3	0	7	3	7.83	18	19
23	4	2	5	1	4	14	5
24	1	0	3	0	9.67	2	5

24	2	10	3	3.5	6.33	1	3
24	3	0	0	0	0	0	0
24	4	1	11	1	11.36	4	1
25	1	0	3	0	18	32	2
25	2	0	0	0	0	47	31
25	3	2	8	1.5	7.125	29	4
25	4	3	8	1	10.375	30	3
26	1	3	2	2.3	6	24	9
26	2	0	3	0	8	25	17
26	3	1	7	1	6.3	22	3
26	4	1	2	1	6	33	8
27	1	4	4	1	7.75	0	5
27	2	2	8	4	8.125	26	0
27	3	1	3	4	13.66666667	35	4
27	4	1	4	4	12.75	48	2
28	1	0	3	0	6	18	15
28	2	0	0	0	0	16	10
28	3	2	2	2	7	24	20
28	4	4	4	4.5	8	10	23
29	1	3	8	2	6.5	16	32
29	2	0	0	0	0	4	0
29	3	0	6	0	15	31	7
29	4	0	6	0	14.7	35	6
30	1	1	0	3	0	16	12
30	2	2	3	1.5	4	14	7
30	3	5	3	6	8.33	9	2
30	4	0	4	0	5.25	3	9
31	1	0	2	0	1	11	4
31	2	0	5	0	10	6	1
31	3	0	5	0	7.4	20	0
31	4	0	3	0	14.33	23	0
32	1	0	0	0	5.4	0	8
32	2	0	2	0	5.4	4	8
32	3	0	3	0	5.4	38	8
32	4	6	2	6.83	5.4	12	8
33	1	2	6	0.31	4.83	11	0
33	2	1	6	0.38	8.5	12	0
33	3	0	7	0	3.57	20	0
33	4	0	4	0	9.25	12	0
34	1	0	3	0	6	0	15
34	2	0	0	0	0	0	17
34	3	0	4	0	5	30	2
34	4	0	0	0	0	41	41
35	1	0	4	0	6.78	12	12
35	2	1	2	3	2	18	14
35	3	4	0	3	0	0	23
35	4	0	6	0	8.3	6	28
36	1	0	3	0	2	0	13
36	2	0	2	0	4	0	12

36	3	0	1	0	3	0	4
36	4	0	2	0	5	0	4
37	1	0	1	0	1	4	12
37	2	0	2	0	2	2	3
37	3	0	1	0	12	4	3
37	4	0	0	0	0	0	2
38	1	3	2	1.6	4.5	24	20
38	2	0	1	0	2	5	3
38	3	0	0	0	0	13	7
38	4	6	1	6	1	27	2
39	1	3	1	1.3	2.5	16	2
39	2	4	10	1.25	4.5	15	12
39	3	7	11	2.5	2.6	22	17
39	4	7	0	1.8	0	7	30
40	1	0	7	0	6.571428571	11	41
40	2	4	0	6.5	0	21	36
40	3	0	2	0	6.5	30	11
40	4	4	2	11.5	16	42	30

Table 3: This table demonstrated the Raw class data for developed fruit, mean number of seeds/fruits, and number of undeveloped fruit which is including (40 replications) performing the experiments on Brassica rapa over a period of 7 weeks.

Date of planting: January 21, 2020
Date of first cotyledon emergence: January 24, 2020
Date of first true leaf formation: January 28, 2020
Date of first flower bud formation: February 4, 2020
Date of first flower opening: February 7, 2020
Date of cross pollination: February 11,12,13 2020
Date of first fruit formation: February 25, 2020
Date of harvesting: March 3, 2020

Table 4: This table demonstrated the diary of plant development including the date of planting, first cotyledon emergence, first true leaf formation, first flower opening, cross pollination, first fruit formation, and harvesting over the period of 7 weeks

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