Heredity of “*Ipomoea nil*”

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People in the Edo period enjoyed modified "*Ipomoea nil*", a Japanese traditional flower, by breeding different types of them. The modified "*Ipomoea nil*" is extinct because of the war and cultural decline. In 2010, one Ipomoea nil which was named variety A came into being. We wanted to know how it was born, so we investigated the number of chromosomes. It was said that variety A came from *Ipomoea multifida*, whose chromosome number is 58. If truly variety A came from it, the chromosome number of variety A would be 58. Observing the chromosome, it has 30 chromosomes in a cell. So we knew that variety A didn't come from *Ipomoea multifida*. Also, using variety A, we made modified *Ipomoea nil*, and compared the shape between the modified one and the usual one. Some unique points are seen in the modified variety A.

1 Background

Do you know *Ipomoea nil*(picture1)?*Ipomoea nil* is a scientific name of flowers.It is familiar to Japanese people because many of us have grown it when we were elementary school students.We researched a definition of *Ipomoea nil*,but there was no definition.Therefore we defined it as having a vine,blooming early in the morning and fading at night,having cone-shaped petals,being gamopetalous flower.

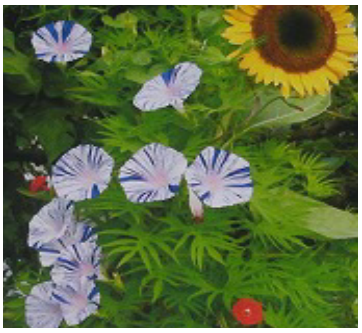
Picture1:*Ipomoea nil*

In 2010,a new species of *Ipomoea nil* was found in a farm.We decided to call the flower variety A(picture2).Variety A is believed to have mutated from *Ipomoea multifida*(picture3).It blooms small red flower,and it has palmate leaves like maple leaves. Besides, it is not *Ipomoea nil* because it is perennial.

Picture2:Variety A

Picture3:*Ipomoea multifida*

There are two features about variety A. First, variety A has the same leaves as *Ipomoea multifida*. Palmate leaves(picture4) are unusual among *Ipomoea nil*.And to my surprise, the next year,it had round leaves(picture5).After that, the leaves of variety A are round,so they were palmate leaves only in the first year.



Picture4: Palmate leaves of variety A

Picture5: Round leaves

Second,it has blue and white stripe petals. Color of *Ipomoea multifida*'s petals is red. Therefore you can see that there is a big change

happening.Besides, the petals of variety A are much larger than the petals of the *Ipomoea multifida*,and it is *Ipomoea nil* because it meets all the requirements.

Next,we explain "*Ipomoea nil* culture" of the Edo period.Edo period lasted from 1603 to 1868.In Edo period,common people enjoyed "*Ipomoea nil* culture" in Edo period.It is to grow a unique *Ipomoea nil*.The unique *Ipomoea nil* is called "henka asagao"."Asagao" means *Ipomoea nil* in Japanese."Henka asagao" is the result of a genetic change during crossbreeding,and it is an *Ipomoea nil* with changing features.People enjoyed growing and protecting "henka asagao" because they have unique petals,leaves or vines.However they were almost lost because of wars and decline of "*Ipomoea nil* culture".

Look at picture6.This picture was painted in Edo period.In other words,it was birthed when "*Ipomoea nil* culture" was prevalent.There are *Ipomoea nil* like variety A in this picture.They have blue and white stripe petals,too.So we researched it because we thought it revived from the Edo period to the present.

Picture6:Painting

First we studied the phylogenetic background of variety A.Then I found out that it looks like diagram1.

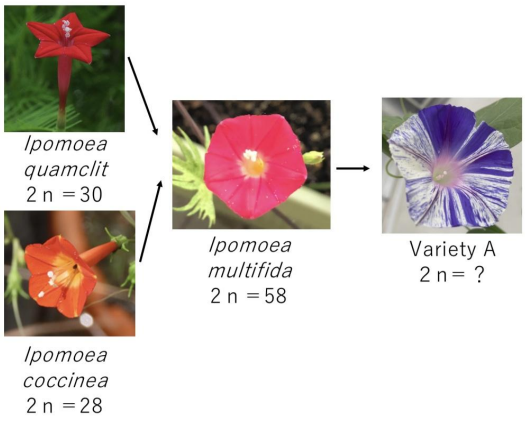


Diagram1:How did variety A come about?

Ipomoea multifida is the result of a cross between an *Ipomoea quamoclit* and an *Ipomoea coccinea*.And variety A is believed to have mutated from *Ipomoea multifida*.Like *Ipomoea multifida*,*Ipomoea quamqlit* and *Ipomoea coccinea* are not *Ipomoea nil* because they are perennials.

In addition, the chromosome number of *Ipomoea multifida* is 2n=58, and the chromosome number of variety A is unknown.If we can determine the number of chromosomes in variety A, it will provide genetic evidence that variety A was mutated from *Ipomoea multifida*. Currently, that photo is the only evidence of mutation, so it would be very important evidence.

The purpose of this study is to determine the number of chromosomes in variety A and to support the mutation of variety A from *Ipomoea multifida*.

2Methods

Experiment1

As mentioned in the background, we examined chromosome numbers to determine the roots of variety A in the experiment 1

. The following are the preparations:

・Colchicine solution(0.05%)
・Carnoy solution
・Acetic orcein
・20ml of cellulase and pectinase solution(=0.2g:0.115g)
・Apical meristems in variety A
・An incubator
・An optical microscope

~What is colchicine?~

Colchicine solution is a chemical that inhibits the formation of mitotic spindles formed during cell division. The mitotic spindle is the structure that segregates chromosomes to two cells during mitosis. By treating the cells with colchicine solution, the mitotic spindle is not formed and twice the original number of chromosomes are identified in the cells. Colchicine is widely used in chromosome observation experiments because it reduces the number of cells that are difficult to count chromosomes due to chromosome aggregation.

If our hypothesis is correct, the number of chromosomes in the cells before the doubling of chromosome number by colchicine should be 58, and the number of chromosomes in the cells after the doubling of chromosome number should be 116.(Diagram2)

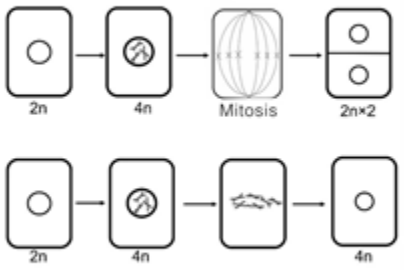


Diagram2:The work of colchicine solution

~Carnoy's solution~

The Carnoy's solution was also used to maintain the colchicine treatment.

The procedure of the experiment 1 is below:

1. Treat colchicine on shoot apical meristem of variety A
2. Fix cells with Carnoy's solution
3. Immerse the shoot apical meristem in water for 2 hours
4. Soak the shoot apical meristem in the solution of cellulase and pectinase
5. Remove the shoot apical meristem from the solution and soak it in water for 2 days
6. Remove unwanted parts of the shoot apical meristem except from the root tip to the root cap
7. Crush the shoot apical meristem with a tweezer while adding Carnoy's solution and dry it
8. Observe it with a microscope

Experiment2

In this experiment, we used variety A to produce mutated Ipomoea nil. The chemical introduced in the background is colchicine, which was used in Experiment 1, and we investigated whether there was a change in the traits of variety A by doubling the number of chromosomes through growth observation. The following preparations are shown.

・Colchicine treated seeds of variety A

・Regular variety A seeds

・Planters, soil, and other cultivation equipment

The colchicine treated strains are referred to as "treated strains" and the colchicine untreated strains are "untreated strains".The following points of the two types of strains were compared. The two types of plants were grown under the same conditions.

・Comparison of growth rates

・Characteristics of cotyledons

・Number of germinating seeds

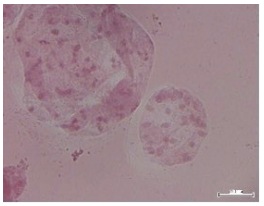
・Characteristics of petals

・Size of petals

3 Results&Discussion

Experiment1

Cells with chromosome numbers of 30 and 60 were identified.(PIcture7)



Picture7:The cell of variety A

From this result, we can say that the number of chromosomes in variety A is 2n=30. Therefore, unlike our hypothesis, variety A is not a mutant from *Ipomoea multifida*.

If variety A is not a mutation from Ipomoea multifida, to which type of flower does variety A belong? Are they morning glories in the first place? In order to solve this question, we are looking forward to studying the base sequence of variety A in the future.

Experiment2

Comparison of growth rates

We compared the number of days from the day the seeds were planted to the day they germinated between treated strains and untreated strains. It took 7 days for the untreated strains and 10 days for the treated ones.The date of planting is different between untreated and treated strains, it may be hard to say, because the difference in sunshine hours may have affected the growth of the plants, considering only the numbers, we can assume that the untreated strains grow faster.(Diagram3)

As a reference for comparison of growth rate, we can see that the treated strains that should have been planted earlier were overtaken by the untreated strains that were planted later in their height.(Picture8)

	The date of planting*1	The date of germination *2	*2-*1
Untreated strains	2/24	3/03	7days
Treated strains	12/08	12/18	10days

Diagram3:Until the seeds germinate

Picture:Strains of variety A
*Left:Treated strains Right:Untreated strains

Characteristics of cotyledons

Observation showed that the cotyledons of the treated strains had thicker leaves and thicker stems than those of the untreated strains.(Picture9)

Picture9:Cotyledons of variety A
*Left:Untreated strains Right:Treated strains

Number of germinating seeds

The untreated strains germinated 9 out of 10 seeds and we are growing 9 plants. The reason why there are only three treated strains is that some of them died after germination. We think this is because *Ipomoea nil* stopped growing as a result of trying to stop the proliferation of cells that did not have the original number of chromosomes, or because the concentration of colchicine was so high that it inhibited growth.(PIcture10)

Picture10:How many the strains are growing

Characteristics of petals

Comparing the characteristics of the petals, the treated strains were found to have incisions not seen in the normal Ipomoea nil and some petals were lighter in color.(Picture11)

Picture11:The petals of untreated strains

Size of petals

The size of the petals was compared by measuring the diameter. In general, the more chromosomes there are, the larger the plant tends to be, but when we compared the average values from the data we have, the untreated strains with fewer chromosomes were slightly larger. Since not enough *Ipomoea nil* has grown to obtain sufficient data, we are still collecting diameter data.(Diagram4)

Untreated strains(cm)	Treated strains(cm)
4	4.5
4	4.5
5	4.5
4.5	3.5
	3.5
Average 4.375	Average 4.1

Diagram4:The diameter of the petals

4Conclusion

In this study, we found out that variety A was not mutated from Ipomoea multifida. Therefore, we would like to clarify which species variety A is related to by studying the base sequence. In addition, we succeeded in collecting seeds from treated strains used in experiment 2. Using these seeds, we would like to confirm whether the chromosome number of treated strains are really doubled or not by the same procedure as in experiment 1. In addition, we will continue to grow and observe treated strains used in experiment 2, because we have not yet obtained sufficient results.

In the future, we would like to find out what kind of Ipomoea nil variety A is. In addition, we would like to clarify the genetic background by examining the number of chromosomes and base sequences of Ipomoea nil that has mutations for which there is no genetic evidence, as in the case of the other Ipomoea nil. I hope that the

mechanism of mutation of Ipomoea nil will be clarified in this way, and that not only ipomoea nil but also mutated ipomoea nil will become horticulture that can be enjoyed by all, as in the Edo period.

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