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STUDIES ON THE BOTANICAL CHARACTERISTICS OF GENUS DIOSCOREA

II. On the formation and germination of the seed in Chinese yam (cv. Nagaimo)

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Introduction

'Nagaimo' is one of the most important cultivar of Chinese Yam (*Dioscorea opposita* THUNB.) in Japan and is cultivated in Aomori, Ibaragi, Nagano, Hokkaido and other prefectures.

In this group, it has been impossible to breed a new cultivar through hybridization because male plants alone are used for cultivation and no female plant is found under ordinary circumstances. In 1972, female plants of 'Nagaimo' were found in Hokkaido prefecture and were multiplied by means of tuber cuttings at Hokkaido University. Since 1973, male and female plants were planted alternately in the field to facilitate open pollination and enhance seed formation. Fruiting of female plants was observed rarely, and seed setting in the fruits was observed more rarely in most years, but in 1978, a large number of fruits were formed by open pollination.

The objective of this paper is to report on a study of the fruiting, seed formation and germination of the 1978 crop.

I. Materials and methods

1. Fruiting and seed formation in 1978.

In 1978, one hundred and fortythree female plants and about two hundred male plants of cv. 'Nagaimo' were planted alternately in the experimental farm of Hokkaido University. Their vines were twined each other on the bamboo supports to facilitate open pollination. In October, all of fruits formed on the female plants were harvested and classified into three size groups. Then seeds were taken out of each fruit and the number

and size of seeds were measured. After that, the seeds were stored at room temperature in a desiccator with silica gel until using for germination experiments.

2. *Seed germination.*

Three kinds of germination experiments were carried out to study the viability of the seeds and the effects of chilling and gibberellins on seed germination.

(A) Viability of seeds harvested in 1978: 20 or 30 seeds were sown on filter paper moistened with water in a 12 cm Petri dish on December 26th, 1978, January 19th, and February 17th, 1979. The dishes were placed in a germinator at 25°C with daily 16-hour periods of illumination (4,000 lux). The germinating seeds were counted daily during the germination period.

(B) Effect of prechilling on seed germination: Seeds were sown the same way as in experiment (A). Prechilling treatments before transfer to higher-temperature conditions were done in a refrigerator at 0°C in the dark for zero (control), four or eight weeks respectively. After that, seeds were kept under the same condition as in experiment (A), and the germinating seeds were counted daily throughout the experimental period.

(C) Effect of gibberellins on seed germination: Seeds were sown on the filter paper moistened with solutions containing 0, 1, 10, 30 and 100 ppm of gibberellic acid and kept in a germinator adjusted to the same conditions as in experiment (A). Counting of germinating and non-germinating seeds was done daily throughout the experimental period.

3. *Aseptic culture of embryos from ungerminating seed*

Embryos of ungerminating seeds in previous experiments were picked out from the seeds and were cultured aseptically on MURASHIGE and SKOOG's medium with 20 g/l sucrose and 7 g/l agar. The cultures were incubated under the conditions of 25°C and illumination 16 hours per day with a 40-watt daylight fluorescent lamp (4,000 lux).

II. Results

1. *Fruiting and seed formation in 1978*

As shown in Table 1, 4,148 fruits large in size, 1,537 fruits medium in size and a large number of fruits small in size were formed on the 143 female plants. The 624 large seeds and many medium and small sized ones were produced in large fruits, but no seed was produced in the medium

TABLE 1. Number of fruits and seeds produced on female plants of 'Nagaimo' in 1978

Marks of female line	Number of plants of each line	Fruits			Seeds		
		Large	Medium	Small	Large	Medium	Small
A	12	39	20	a large number	5	6	a large number
B	12	361	210	"	76	82	"
C	36	1,420	408	"	156	251	"
F	21	871	400	"	141	195	"
H	10	329	88	"	39	63	"
J	27	383	105	"	107	97	"
N	25	745	306	"	100	155	"
Total	143	4,148	1,537	"	624	849	"

* Fruit {Large: >1.5 cm in diameter, Medium: 1.0 to 1.5 cm in diameter,
 {Small: <1.0 cm in Diameter

Seed {Large: >5 mm in diameter, Medium: 3 to 5 mm in diameter,
 {Small: <3 mm in diameter

** Diameter of seeds exclude the width of wing.

and small sized fruits.

Concerning seed size, differences were observed not only in diameter but also in thickness, and embryos which seemed to be matured enough to germinate were observed only in the seeds 1 mm or more in thickness and 5 mm or more in diameter.

On the contrary, although fruiting was observed in 1972, the number of mature seeds harvested were only 24. No more, and usually less seed than those in 1972 were formed in each year of 1973-1977.

2. Seed germination

(A) Viability of seeds harvested in 1978: As shown in Table 2, seed germinated in three tests, but the germination percentage in these tests was

TABLE 2. Germination of the seeds produced in 1978

Test-starting date	Days after transfer to 25°C			Total	Germination percentage (%)
	10	20	30		
December 26th, 1978	3/30	4/30	0/30	7/30	23.3
January 19th, 1979	0/20	1/20	1/20	2/20	10.0
February 17th, 1979	0/20	2/20	0/20	2/20	10.0

very low. Namely, in the 1st test in December, seed germination was observed after 10 days of incubation at higher temperature, and the germination percentage after 30 days of incubation was 23.3. In the 2nd test in January and 3rd test in February, 1979, seeds started to germinate after 20 days of incubation and germination percentage after 30 days of incubation was only 10.

(B) Effect of chilling on seed germination: Germination was enhanced remarkably by chilling treatment, and the germination of the seeds increased with an increasing of prechilling period (Fig. 1). Namely, 10 percent of germination percentage was shown in control without chilling, whereas the germination percentage was 20 percent in the treatment of 4 week-chilling, and 40 percent in the treatment of 8 week-chilling.

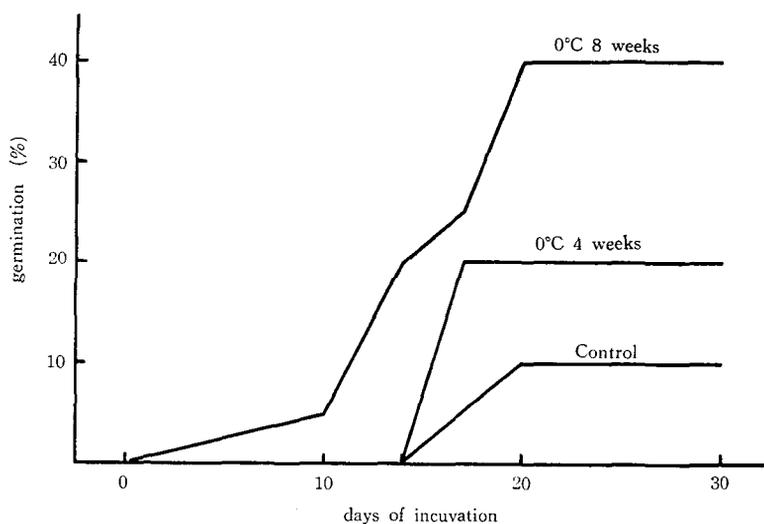


Fig. 1. Effect of the prechilling at 0°C on germination of *D. opposita* (cv. Nagaimo) seeds.

Seeds prechilled at 0°C in the dark for 4 or 8 weeks were incubated at 25°C.

(C) Effect of gibberellins on seed germination: As shown in Fig. 2, the effectiveness of gibberellins (GA_3) on seed germination was not distinct. However in the presence of 1 and 30 ppm of GA_3 , the germination percentage increased by ca. 5 to 10 percent compared with that of the control.

3. Aseptic culture of embryo from ungerminating seed

Almost 100 percent of the embryos picked out from the ungerminating

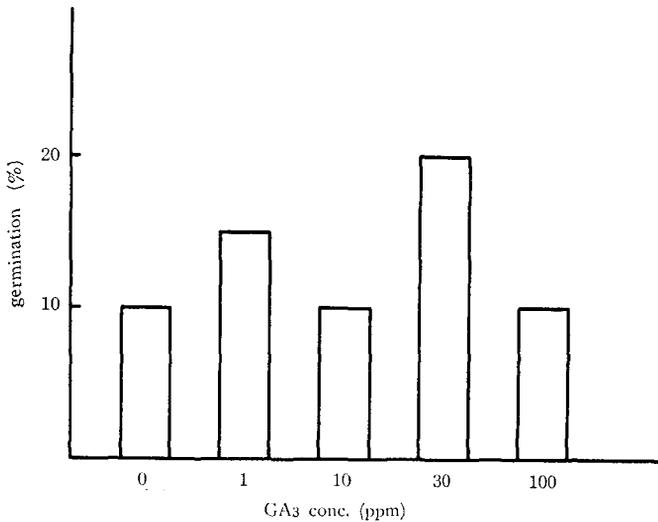


Fig. 2. Effect of GA₃ on germination of *D. opposita* (cv. Nagaimo) seeds.

Seeds which had been stored for about 2 months at room temperature were incubated at 25°C.

seeds were grown into normal plants by embryo culture. The growth pattern of the embryos cultured *in vitro* was similar to the pattern in normal seedling growth as shown in Plate I. Namely, at first the primary root elongated into the agar (A-C) and then the first leaf appeared on the basal part of the cotyledon (D). Elongation of the stem was observed within three weeks after the beginning of culture (E) and the cotyledon stayed at the basal part of the stem (F). The plantlets developed by embryo culture were transplanted to pots after about 1 month of culture. The growth of these plantlets was like that of seedlings developed by normal germination. After one growing season, various types of tubers were observed.

Discussion

In Japan, three cultivars of Chinese yam (*Dioscorea opposita*) are usually cultivated. The names of these cultivars are 'Nagaimo', 'Ichoimo' and 'Yamatoimo'. Concerning sex expression, only male plants are found in cv. 'Nagaimo' and only female plants are found in cv. 'Ichoimo' and 'Yamatoimo' under ordinary circumstances.

In 1954, MATUO and MIZUNO reported that a few fruits were formed on bisexual plants of 'Yamatoimo' by self pollination, however, no mature

seed was formed^{2,3)}. AOBA succeeded in harvesting 636 seeds by crossing 'Ichoimo' (♀) × 'Nagaimo' (♂) and produced 2 seedlings from these seeds the following year¹⁾. These are all of the reports published previously relating to the production and germination of seeds of *Dioscorea opposita* and there have been no papers on the seed of 'Nagaimo' except for the previous paper⁶⁾.

Since 1972, seed setting of 'Nagaimo' was observed every year, and in 1978 a great number of seeds was harvested through natural crossing (Table 1). The reason why so many seeds were produced in 1978 alone is not clear, but one of the reasons for excellent seed setting could be that it was unusually hot and dry in summer in that year.

From the results of experiments on seed germination, light dormancy was observed in the seeds of 'Nagaimo' and the dormancy of these seeds was broken somewhat by chilling or gibberellic acid (GA₃) treatments (Fig. 1, 2); the highest percentage of germination was 40.0 with chilling treatment. The dormancy of the seed of *Dioscorea* was studied in detail by OKAGAMI and KAWAI using two species which set seed easily^{4,5)}. Namely, they reported that the seeds of *Dioscorea tokoro* MAKINO and *D. tenuipes* FRANCH. et SAVAT. have a dormancy so that for complete germination, seeds of both species required prechilling under moist conditions before incubation at a higher temperature. In addition, the interesting results such as the effects of light and gibberellic acid application on the germination were described in their papers. We wanted to examine the function of these factors too, but it was impossible, because the number of seeds harvested was not enough to carry out various experiments in detail.

Embryos of the ungerminating seeds in these experiments were cultured *in vitro* with the technique of embryo culture and almost all of embryos were grown into normal plantlets. In the near future, the sex ratio, chromosome number, and other botanical characteristics of these seedlings will be observed.

Summary

In 1972, female plants of 'Nagaimo' were found and were multiplied by means of tuber cuttings at Hokkaido University. In this paper, fruiting, seed formation, and the germination of the seeds were observed. The experimental results are summarized as follows:

1. Fruiting of female plants was observed rarely, and seed setting in the fruits was rare in ordinary years, but in 1978 alone, a large number of fruits and seeds were formed by open pollination. The reason so many

seeds were produced in 1978 alone is not clear, but one possibility could be that it was unusually hot and dry in the summer in that year.

2. Light dormancy was observed in the seeds of 'Nagaimo' and the dormancy of these seeds was broken somewhat by chilling or gibberellic acid (GA₃) treatments, and the highest percentage of germinating seed was 40.0 with chilling treatment.

3. Embryos of the ungerminating seeds in these experiments were cultured *in vitro* with the technique of embryo culture and almost all of embryos were grown into normal plantlets.

In the near future, the sex ratio, chromosome number, and other botanical characteristics of these seedlings will be observed.

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Explanation of Plate

- A : Embryo picked out from the endosperm of seed.
- B : Embryo on the culture medium.
- C : Embryo elongating root.
- D : Root and first leaf elongation.
- E : Seedling grown by embryo culture.
- F : Cotyledon stayed at the base of stem.

