Some Characteristics of Interspecific Hybrids between *Dioscorea japonica* Thunb. and *Dioscorea opposita* Thunb¹.

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Summary

In 1975, we obtained 2 seeds which appeared to be hybrids from a natural cross between *D. japonica* and *D. opposita* (cv. Nagaimo). Two seedlings were produced from them by embryo culture. In 1980, chromosome number, sex, structure of flowers, shape of leaves and underground tubers and quality of tubers of the 2 plants were investigated. The results obtained are summarized as follows.

1. Both the 2 plants showed the chromosome number of 110 and proved to be hybrids between *D. japonica* ($2n=80$) and *D. opposita* ($2n=140$).

2. The 2 hybrids were both male plants and the morphological structure of flowers was similar to that of *D. opposita*, but pollen fertility was lower than that of *D. opposita*.

3. The tubers of the hybrids were as long as those of *D. opposita*, intermediate in thickness between those of the parents and more mucilaginous than those of *D. opposita*.

Thus, a possibility was suggested to get new *Dioscoreas* with superior characteristics by hybridization between *D. japonica* and *D. opposita*.

Introduction

Dioscorea opposita cv. Nagaimo is cultivated widely in Japan, and its yield per unit area is high, while its textural qualities are inferior to those of other cultivars of the same species such as 'Yamatoimo' and 'Ichioimo'. On the other hand, *D. japonica* (Jinenjo in Japanese) has excellent qualities, but its tuber is not so thick as that of *D. opposita* (This scientific name denotes hereafter cv. Nagaimo alone) and the yield is low. In addition, a kind of virus infecting *D. opposita* does not infect *D. japonica*. No report has been published concerning the hybridization between *D. japonica* and *D. opposita*.

The female plants of *D. japonica* and male plants of *D. opposita* have been planted alternately with each other in the experimental field of Hokkaido University in Sapporo since

1972. The female plants of *D. japonica* were collected at Gonohe in Aomori Prefecture and the male plants of *D. opposita* were collected at Yubari in Hokkaido. In 1975, 2 seeds were obtained from a female plant of *D. japonica*. These 2 seeds were considered to be hybrids between *D. japonica* and *D. opposita* because there was no male plant except those of *D. opposita* in the field. In 1976, 2 seedlings were obtained from these seeds by embryo culture, and proved to be hybrids according to the evaluation of their chromosome numbers. In the present paper, they are designated as Hybrid 1 and Hybrid 2, and some of their characteristics were investigated in comparison with those of *D. japonica* and *D. opposita*.

Materials and Methods

From 1977 to 1979, the tubers of Hybrid 1 and Hybrid 2 were divided into 12 and 9 pieces, respectively. In April 1980, these pieces were germinated in a green house and in June, they were planted in a field at

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Yubari in Hokkaido—one of the producing centers of *D. opposita*. In Yubari, volcanic ash soil prevails. It is so loose and deep that the tubers can elongate free of physical obstacles, and it seemed to be suitable for investigation of the shape of tubers.

At sprouting, the chromosome number was observed with root tips to confirm whether or not these plants are hybrids between *D. japonica* and *D. opposita*. In the summer, the sex and the characteristics of flowers and leaves were investigated. The tubers were harvested on November 5 and their shapes were observed. The details of methods used for observations were described below.

1. Chromosome number

The tubers were exposed to 0°C for 3 months to break the dormancy. Thereafter, they were divided into seed pieces about 50 g in weight and planted in a rooting bed. When they rooted after 3 weeks, the adventitious root tips were used for observation of the chromosome. For that purpose, the root tips were kept at 0°C for 24 hours and then fixed in Östergren and Heneen's fluid for 24 hours(9). They were stained with aceto-carmin and observed by the squash method.

2. Characteristics of flowers and leaves

On August 24, the flowers were collected and fixed in FAA. The structure of flowers was observed with paraffin sections stained with Mayer's hemalum. Pollen fertility was determined by staining with acetocarmin. Observation of leaves was carried out using the 15th or upper leaves from the plant

Table 1. Comparison of pollen fertility among Hybrid 1 and 2 and their male parents, *D. opposita*.

Line	Pollen fertility (%)
<i>D. opposita</i>	94.8 a ^z
Hybrid 1	81.5 b
Hybrid 2	61.7 c

^z Mean separation by Duncan's new multiple range test, 5% level.

base, because they were less variable in shape, color and luster.

3. Characteristics of underground tubers

The length, diameter and weight of tubers and the appearance of their surface were investigated. The water content and the mucilage of the flesh of tubers were also investigated for quality. Ten g of grated tuber was dried at 70°C for 24 hours in a drying chamber equipped with a fan. Then the weight of the dry matter was measured and water content was calculated. Fifty g of tuber blocks were homogenized together with 50 ml of water for 30 seconds. After leaving for 1, 5, 10 and 20 minutes, 15 g of the homogenate was dropped through a glass tube (1 m long having 6 mm bore) and the time required for the downward flow of homogenized sample through the tube was recorded in minutes.

Results

1. Chromosome number

All chromosomes observed in this study were very small and granular ranging from

Fig. 1. Comparison of chromosome number.

A : *D. opposita*, male ($2n=140$),

B : *D. japonica*, female ($2n=80$),

C : hybrids between *D. japonica* and *D. opposita* ($2n=110$).

Fig. 2. Structure of the male flower of hybrid (Hybrid 1) between *D. japonica* and *D. opposita*.
A : vertical section, B, C : cross section,
a : anthers and pollens, f : filaments,
dp : degenerated pistil,

Fig. 3. Comparison of the shape of leaves.
A : *D. opposita*, B : *D. japonica*,
C : Hybrid 1, D : Hybrid 2.
a : angle of the sinus on leafbase,
b : angle of the rounded sinus on margin.

1 to 1.5 μm in length. The chromosome number was 140 in the male plant of *D.*

opposita and 80 in the female plant of *D. japonica* (unpublished), while it was 110 in the 2 plants newly obtained (Fig. 1). From this result, these 2 plants proved to be hybrids between *D. japonica* and *D. opposita*.

2. Characteristics of the flowers and leaves

Both Hybrid 1 and 2 were male plants and developed male flowers alone. The flowers were similar in shape to those of *D. opposita* (Fig. 2). The flowers of these hybrids were larger in size than those of *D. opposita*. Thus, the male flowers of *D. opposita* were 2.5 to 3.0 mm in width, whereas those of Hybrid 1 and 2 were 3.7 and 3.3 mm in width, respectively.

The pollen fertility was significantly lower in the hybrids than *D. opposita* (Table 1).

The typical shapes of leaves observed are shown in Fig. 3. The leaf of *D. opposita* is deeply cordate with rounded sinus and acuminate at the top of the leaf, while that of *D. japonica* is simple and acuminate. The leaf shape of *Dioscorea* can be characterized by the angles of sinus on the leaf base (Fig. 3-a) and on the leaf margin (Fig. 3-b). In Hybrid 1, the former (Fig. 3-a) was small as

Fig. 4. Comparison of the shape of underground tubers
A : *D. japonica*, B : *D. opposita*, C : Hybrid 1, D : Hybrid 2.

Table 2. Comparison of some characteristics of underground tubers among Hybrid 1 and 2 and their parents.

Character	Line			
	<i>D. opposita</i>	<i>D. japonica</i>	Hybrid 1	Hybrid 2
Length (cm)	50~70	60~100	60~80	40~60
NL/TL ^z	0.22~0.40	—	0.29~0.40	0.22~0.33
Diameter of tuber(cm)	5.0~8.0	1.5~3.0	2.5~5.0	2.5~5.0
TD/ND ^y	2.0~5.0	1.0	1.5~3.0	2.0~3.5

^z Ratio of neck length to tuber length.^y Ratio of tuber to neck in diameter.

in *D. opposita* and the latter (Fig. 3-b) was large as in *D. japonica*. On the other hand, in Hybrid 2, the former was large as in *D. japonica* and the latter was small as in *D. opposita*. The leaves of Hybrid 1 and 2 were both waxy dark green, and 0.28 and 0.30 mm in thickness respectively, being as thick as that of *D. opposita*.

3. Shape of underground tubers.

The shapes and some characteristics of underground tubers are shown in Fig. 4 and Table 2, respectively. The tuber of *D. opposita* had a slender neck which could be distinguished clearly from the other part. But in *D. japonica*, the tuber was uniformly slender throughout its length and the neck was not clearly distinguished. While in the hybrids, the neck could not be easily distinguished from the other part and the ratio of it to the whole tuber in length differed little from that of *D. opposita*. The diameter of tuber in the hybrids was smaller than that of *D. opposita* but larger than that of *D. japonica*. The tubers of the hybrids were heavier than those of *D. japonica* and as heavy as those of *D. opposita*. Especially in Hybrid 1, one of the largest tubers weighed as much as 930 g. The tubers of Hybrid 1 showed a tendency

to be more slender than those of Hybrid 2. The tubers of the hybrids were round or sharp at their tips. That of *D. opposita* was round, while that of *D. japonica* was sharp at the tip. The surface of the tubers was more rough in the hybrids than *D. opposita* and *D. japonica*.

4. Water content of underground tubers

The water content of underground tubers

Table 3. Comparison of water content in underground tubers among Hybrid 1 and 2 and their parents.

Line	Water content (%)
<i>D. opposita</i>	80.3 a ^z
<i>D. japonica</i>	64.5 b
Hybrid 1	61.8 bc
Hybrid 2	67.8 c

^z Mean separation by Duncan's new multiple range test, 5% level.

Fig. 5. Comparison of the mucilage of the flesh of tubers.

(○) *D. opposita*, (●) *D. japonica*,
(△) Hybrid 1, Vertical lines represent SD
(▲) Hybrid 2.

of the hybrids was similar to that of *D. japonica* and much lower than that of *D. opposita* (Table 3).

5. Mucilage of the flesh of underground tubers

The homogenate of flesh of *D. opposita* was watery and 15 g of it flowed down through the glass tube in 5 to 6 seconds, while that of *D. japonica* required 8 to 22 minutes because of high mucilage. In contrast, in the case of the hybrids, the time required was intermediate between *D. opposita* and *D. japonica* (Fig. 5). It was confirmed that in Hybrid 1 and 2, the flesh of the tubers was more mucilaginous than *D. opposita* but not so mucilaginous as *D. japonica*.

In *D. opposita*, the time required for the downward flow of the homogenized sample through the glass tube was almost constant regardless of the time after homogenization. While in *D. japonica* and the hybrids, it increased with time after homogenization (Fig. 5).

Discussion

In the parent plants and their hybrids, the chromosome numbers were all multiples of 10 as reported for Old World species (3, 4, 5, 6, 7, 8, 11, 12, 13). As in other species of *Dioscorea*, karyotype analysis was unsuccessful because the chromosomes observed were too small. It was confirmed that the 2 plants obtained were hybrids between *D. japonica* and *D. opposita*, because their chromosome number was 110, which is the mean of 80 in *D. japonica* and 140 in *D. opposita*.

As described in the introduction, both *D. japonica* and *D. opposita* have some merits and some demerits as cultivated species. The hybrids were intermediate between the parents with respect to the shape and quality of tubers. This result indicates a possibility to improve demerits of each species through hybridization.

As to disease resistance to viruses, about 20% of *D. opposita* were infected with some kind of viruses, but no symptoms due to viruses were observed in the hybrid plants grown in the same field. This phenomenon

is noticeable and whether or not the hybrids are infected with the viruses which attack *D. japonica* is now under investigation.

Until now, the breeding of *Dioscorea* has been carried out mainly by selection of the plants with excellent characteristics in connection with yield, keeping quality and so on. Some trials have been reported on the breeding of yam through hybridization (1, 2, 10). The purposes of these studies were to increase the yield of tubers and the content of steroidal sapogenin. In 1966, Martin *et al.* partially succeeded in crossing within the New World species and obtained hybrids from 13 combinations, but they always failed in crossing between New and Old World species or within Old World species (2). Rao *et al.* found out 3 combinations within New World species which produced seeds with normal germination ability (10). In this experiment, we succeeded in crossing between 2 Old World species, *D. japonica* and *D. opposita*. The hybrids obtained by Martin *et al.* were intermediate between their parents in some characters of vegetative organs and reproductive organs and they exhibited poor physiological control of critical growth phases such as the juvenile phase. Our hybrids were intermediate between their parents in some characters of vegetative organs as in the case of Martin *et al.*, but not in reproductive organs. Thus, the male flowers of Hybrid 1 and 2 were larger than those of *D. opposita*.

In general, it is difficult, but not impossible, to obtain hybrid seeds when *D. japonica* and *D. opposita* are planted alternately with each other and crossed by open or artificial pollination. In fact, in 1975, 2 seeds were obtained by open pollination. In breeding of *Dioscorea*, it is important to clarify the environmental conditions enhancing the seed formation.

Fortunately, many hybrid seeds were obtained between *D. japonica* and *D. opposita* by open pollination in 1978. The plants with wide variations in some characters are expected to be obtained from these seeds.

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ジネンジョとヤマノイモとの種間雑種の特性について

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摘 要

1975年にジネンジョ(♀)とヤマノイモ(‘ナガイモ’ ♂)との自然交配で2個の種子が得られ、胚培養により2株の実生を育成した。これらの実生を、ウイルスなどに感染しないように隔離栽培し、切り芋法により増殖させた。1980年には着花し、地上部および地下部においてそれぞれ特徴ある形状を示したので、種々の特性を両親との比較で調査した。

- 実生の染色体数は $2n=110$ で、ジネンジョ ($2n=80$) と ‘ナガイモ’ ($2n=140$) の中間の数で、染色体数の上から両種の種間雑種であることが確認された。
- 雑種実生は2株とも雄株で、‘ナガイモ’と同じ構

造の雄花を着生したが、花粉稔性は‘ナガイモ’のそれより低かった。

- 芋の形状は‘ナガイモ’程度の長さになり、ジネンジョよりもはるかに太くなった。また、水分含量や粘りなどの品質に関してジネンジョと‘ナガイモ’の中間的な特徴を示し、‘ナガイモ’より水分が少なく、粘りも強くなった。このように雑種実生は、形態と品質の両面において、ジネンジョと‘ナガイモ’の短所とされる点が改善されており、今後ヤマノイモ属の栽培種の育種を進める上で極めて注目される。