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Histological Studies on the Relationship between the Process from Fertilization to Embryogenesis and the Low Seed Set of Sweet Potato, *Ipomoea batatas* (L.) Lam.

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To determine the direct cause of the low seed set in sweet potato, 14 cross-compatible combinations among 11 varieties were histologically examined for the process from fertilization to embryogenesis at 4, 7 and 48 hours after pollination. Pollen fertility on the flowering day exceeded 80% in most of the varieties examined and pollen germination 4 hours after pollination reached 10~20% in most of the crosses. These results show that neither the fertility of mature pollen nor the pollen germination on stigma is the principal cause of the low seed set. The mean number of pollen tubes passing through the conducting tissue of the ovary 7 hours after pollination largely differed among the cross combinations. However, it did not show a close relationship with the number of seeds per capsule. Among the embryosacs examined 48 hours after pollination, those that had not been penetrated by pollen tubes were mostly abnormal, in which the development of the egg apparatus had been interrupted halfway. On the other hand, the number of normal young embryos was closely correlated with the number of seeds per capsule. These results suggest that a large number of such abnormal embryosacs is the principal cause of the low seed set of sweet potato.

Key Words: ***Ipomoea batatas*, low seed set, pollen fertility, pollen germination, pollen tube, abnormal embryosac.**

Introduction

Many investigations have been carried out to determine the factors affecting the general low seed set observed in sweet potato breeding. These investigations have shown that the self- and cross-incompatibility existing in many clones of this crop undoubtedly restricted the seed set (Wada 1923, Terao 1934, Togari and Kawahara 1946a, 1946b, Shigemura 1943, Hernandez and Miller 1962, 1964, Fujise 1964, Wang 1964, Martin 1965, 1968), but the causes of the low seed set in compatible cross pollination have not been elucidated. Togari and Kawahara (1946a, 1946b) reported on the association of the percentages of germinated pollen and the growth rates of pollen tubes with the rate of capsules set and the number of seeds per capsule among different compatible matings, while, Martin and Ortiz (1965) suggested that factors other than the number of germinated pollen grains also restricted the seed set in sweet potato. Ting and Kehr (1953) reported that a low seed set in some lines of sweet potato might have resulted from meiotic abnormality. However, Jones (1965) who failed to an association of the seed set with the meiotic abnormality, suggested that the low seed set generally observed in sweet potato must be due to causes other than meiotic abnormality, such as diseases, genetically controlled incompatibility or sterility or physiological dysfunction in the developing seed.

In the previous paper, the authors reported the normal process of fertilization and embryogenesis in sweet potato (Kokubu *et al.* 1982). The present study was designed to determine what process in fertilization or embryogenesis was directly responsible for the low seed set in compatible mating in sweet potato.

Materials and Methods

Eleven sweet potato clones belonging to different cross-incompatible groups, A, B, C, E and J, respectively, as shown Table 1 and Table 2, were used in this experiment. The present study was carried out twice (Experiment 1 and Experiment 2).

For flower induction, the stems were grafted onto the dwarf type morning glory, cv., Kidachi-Asagao. The grafted plants were grown in a greenhouse in the temperature range from 15°C to 30°C, which is favorable for the seed set of sweet potato (Fujise 1964, Wang 1964). Artificial pollination was carried out before 10:00 am on flowering days by the following method; 2 stigmas were carefully pollinated with 1 dehiscing anther so that, as much as possible, a similar number of pollen grains was placed on the stigma.

The percentage of capsules set to flowers pollinated and the mean number of seeds per capsule 30 days after pollination were calculated as follows : [(Number of capsules / Number of flowers pollinated) × 100] and [(Number of seeds / Number of capsules) × 100], respectively. Aceto-carmine was used to determine the pollen fertility on flowering days. After rinsing in 1N sodium hydroxide solution was performed for a few seconds, pollen germination on the stigmas and pollen tubes penetrating the upper style 4 hours after pollination were observed after staining with 0.1% aniline blue fluid (a mixture of 10 ml non-fluorescent glycerin, 90 ml

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Table 1. Numbers of capsules per flower pollinated and seeds per capsule (Experiment 1)

No.	Cross combination		Number of flowers pollinated	Number of capsules per flower pollinated	% of capsules to flowers pollinated[1)]	Frequency of capsules with[2)]				Mean number of seeds per capsule[3)]
	Female	Male				1 seed	2 seeds	3 seeds	4 seeds	
1	Kyushu No. 58 (C)	Chikei682-11 (A)	52	44	84.6	11	19	12	2	2.11
2	Chikei682-11 (A)	Kyushu No. 58 (C)	89	71	79.8	18	35	15	3	2.04
3	Kyushu No. 58 (C)	F683-4 (B)	60	48	80.0	15	22	11	0	1.91
4	F683-4 (B)	Kyushu No. 58 (C)	29	3	10.3	3	0	0	0	1.00

(A~C): Cross-incompatible group

1) (Number of capsules/Number of flowers pollinated) × 100

2) Frequency of occurrence of capsules with 1,2,3,or 4 seeds

3) (Number of seeds /Number of capsules) × 100

Table 2. Numbers of capsules per flower pollinated and seeds per capsule (Experiment2)

No.	Cross combination		Number of flowers pollinated	Number of capsules per flower pollinated	% of capsules to flowers pollinated[1)]	Frequency of capsules with[2)]				Mean number of seeds per capsule[3)]
	Female	Male				1 seed	2 seeds	3 seeds	4 seeds	
1	Kyushu No. 58 (C)	Chikei682-11 (A)	108	82	75.9	38	28	14	2	1.76
2	Chikei682-11 (A)	Kyushu No. 58 (C)	115	69	60.0	26	30	12	1	1.83
3	Hayato (B)	Kyushu No. 58 (C)	93	53	57.0	33	18	2	0	1.42
4	Tsurunashigenji (A)	Kyushu No. 58 (C)	49	30	61.2	9	14	6	1	1.97
5	Minamiyutaka (B)	Kyushu No. 58 (C)	63	31	49.2	20	9	2	0	1.42
6	Koganesengan (B)	Kyushu No. 58 (C)	55	42	76.4	14	15	12	1	2.00
7	Norin No. 2 (B)	Kyushu No. 58 (C)	86	45	52.3	26	16	3	0	1.49
8	Benikomachi (E)	Kyushu No. 58 (C)	43	21	48.8	15	5	1	0	1.33
9	Kokei No. 14 (E)	Kyushu No. 58 (C)	29	13	44.8	7	5	1	0	1.54
10	FV 62-41 (J)	Kyushu No. 58 (C)	29	17	58.7	16	1	0	0	1.06

(A~J): Cross-incompatible group

1) (Number of capsules/Number of flowers pollinated) × 100

2) Frequency of occurrence of capsules with 1,2,3,or 4 seeds

3) (Number of seeds /Number of capsules) × 100

H_2O, 710 mg K_3PO_4 and 100 mg aniline blue) by fluorescence microscopy. The materials for anatomical observation of the pollen tubes penetrating the conducting tissue of the ovary and embryosac were collected at 7 hours and 2 days after pollination, respectively. The collected materials were fixed in F.A.A.fluid (a mixture of 50% ethanol, formalin and acetic acid, 90 : 5 : 5), and cut transversely or longitudinally into 15 μm thick sections by the ordinary paraffin method and stained with Safranin O and Fast green.

Results

Numbers of capsules per flower pollinated and seeds per capsule

The numbers of capsules per flower pollinated and seeds per capsule are shown in Table 1 and Table 2. The percentage of capsules to flowers pollinated in these combinations ranged from 50% to 80%, except for 10.3% in F683-4 × Kyushu No. 58, and differed with the combinations of crosses as in conventional practices. The mean number of seeds per capsule ranged from 1 to 2. Although the mean number of seeds per capsule in these crossings was too small relative to the ovule number per ovary, 4, many investigators have obtained the same results from compatible crossing of this crop until now.

Pollen fertility on flowering days

Pollen fertility on flowering days exceeded 80% in most of the varieties examined, regardless of the varieties (Table 3). Pollen fertility of sweet potato varieties used in this study was high, indicating that pollen fertility was not the cause of the low seed set.

Pollen germination on stigma and number of pollen tubes penetrating the upper style

Approximately 1 hour after pollination, the pollen grains germinated on the stigma. Pollen germination on the stigma 4 hours after pollination is shown in Table 4 .

In most of the cross combinations, the pollen germination rate 4 hours after pollination was 10~20%, and the number of pollen grains that germinated per flower was also about 10, except in the case of Norin No. 2 × Kyushu No. 58.

The rate of the change in the number of pollen tubes on the stigma to that on the upper style is shown in Table 5. The

Table 3. Pollen fertility on flowering day (Experiment 2)

Variety	Number of flowers observed	Number of pollen grains observed	Number of fertile pollen grains	Pollen fertility (%)
Chikei 682-11	10	1,031	897	85.3
Kyushu No. 58	9	1,188	1,037	87.3
Hayato	11	1,491	1,099	73.7
Minamiyutaka	13	1,874	1,585	84.6
Koganesengan	7	954	871	91.3
Benikomachi	2	223	186	83.4
Kokei No. 14	10	1,755	1,531	87.2
FV 62-41	10	1,287	1,163	90.4

reduction rate in the number of pollen tubes on the stigma to that on the upper style was about 50%, except for about 25% in Norin No. 2 × Kyushu No. 58 and FV62-41 × Kyusyu No. 58. About 50% of the pollen tubes observed on the stigma failed to penetrate the upper style.

However, many more pollen grains germinated on the stigma and pollen tubes penetrating the upper style in all the cross combinations, compared with the ovule number per ovary, 4 (Fig. 1A). These results indicate that pollen germination on the stigma and pollen tubes penetrating the upper style was not the principal cause of the low seed set.

Number of pollen tubes that penetrated the conducting tissue of an ovary

As states in the previous paper, 4 hours after pollination, the tip of the pollen tube passed through the micropyle and penetrated into the embryosac, and the traces of pollen tubes that passed through the conducting tissue of the ovary could be easily detected at the cross-sections of the tissue, because they either stained red with Safranin O or they were recognizable as small holes in the conducting tissue (Fig. 1B) (Kokubu *et al.* 1982). The rate in the change of the number of pollen tubes that penetrated the upper style to that penetrating the conducting tissue of the ovary is shown in Table 5.

The reduction rate was divided roughly into the following 3 trends, about 10% (Kyushu No. 58 × Chikei 682-11, Norin No. 2 × Kyushu No. 58, Kokei No. 14 × Kyushu No. 58, FV62-41 × Kyushu No. 58), 20~40% (Hayato × Kyushu No. 58, Tsurunashigengi × Kyushu No. 58, Koganesengan × Kyushu No. 58, Benikomachi × Kyushu No. 58) and about 60% reduction rates (Chikei 682-11 × Kyushu No. 58, Minamiyutaka × Kyushu No. 58).

The frequency distribution of the number of pollen tubes penetrating the conducting tissue of the ovary at 7 hours after pollination is shown in Table 6 and Table 7. In the case of Kyushu No. 58 × Chikei 682-11, Chikei 682-11 × Kyushu No. 58 and Kyushu No. 58 × F683-4, the percentage of the frequency of the number of pollen tubes which was about 4, and correspondes to the number of ovules per ovary, showed relatively high values, 75.0, 100 and 87.5, respectively, whereas in the case of F683-4 × Kyushu No. 58, it showed an extremely low value, 12.9 (Table 6). In the cross combinations shown in Table 7, the percentage of the frequency of the number of pollen grains not less than 4 ranges from 10% to more than 90%.

The author observed a relationship between the variety used in the cross and the mean number of pollen tubes penetrating the conducting tissue of the ovary. In Kyushu No. 58 × Chikei 682-11, Kyushu No. 58 × F683-4 and its reciprocal crossing, the mean number of pollen tubes penetrating the conducting tissue depended on the mother parent in each crossing (Table 6). The mean number of pollen tubes penetrating the conducting tissue in Kyushu No. 58 × F683-4 was 5.4, while its reciprocal crossing showed an extremely low value, 1.7. Furthermore, in the cross combinations shown in Table 7, the male parent of all the cross combinations was Kyushu No. 58 , except for Kyushu No. 58 × Chikei 682-11. Therefore, based on the observations, the mean number of pollen tubes penetrating the conducting tissue clearly depended on the female parent and not on the male parent. Al-

Table 4. Pollen germination on flowering day (Experiment 2)

Cross combination		Number of flowers pollinated	Number of pollen grains pollinated per flower	Number of pollen grains which germinated per flower	Pollen germination rate (%)[1)]
Female	Male				
Kyushu No. 58 (C)	Chikei682-11 (A)	14	61.1	10.8	18.2±3.4
Chikei682-11 (A)	Kyushu No. 58 (C)	14	120.0	13.6	11.4±3.7
Hayato (B)	Kyushu No. 58 (C)	14	72.7	11.0	17.7±9.4
Tsurunashigenji (A)	Kyushu No. 58 (C)	12	79.2	8.8	11.5±5.2
Minamiyutaka (B)	Kyushu No. 58 (C)	13	83.0	11.8	16.4±9.0
Koganesengan (B)	Kyushu No. 58 (C)	12	91.5	9.8	10.8±4.7
Norin No. 2 (B)	Kyushu No. 58 (C)	12	68.9	4.7	7.4±4.0
Benikomachi (E)	Kyushu No. 58 (C)	5	70.2	11.2	16.0±14.1
Kokei No. 14 (E)	Kyushu No. 58 (C)	14	90.1	11.6	14.0±6.3
FV 62-41 (J)	Kyushu No. 58 (C)	10	92.3	10.9	14.6±12.2

(A~J): Cross-incompatible group

1) Mean±Standard deviation

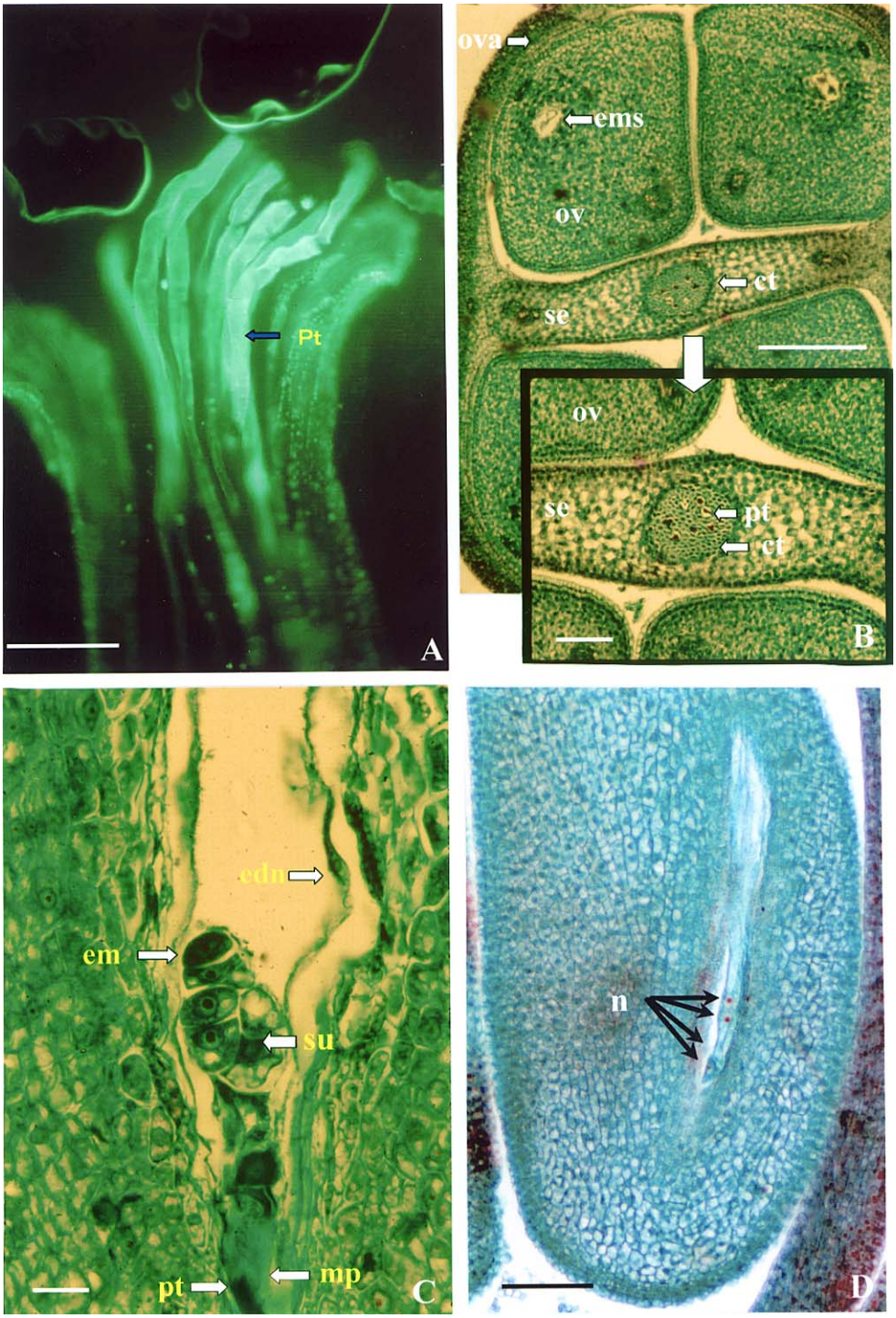

Fig. 1. Histological section showing the process from fertilization to embryogenesis. (A) Pollen tubes penetrating the upper style 4 hours after pollination (×40). (B) Cross-section of the conducting tissue of the ovary 7 hours after pollination(×40, ×100). (C) Longitudinal section of normal developing embryo 2 days after pollination (Chikei 682-11×Kyushu No. 58) (×100). (D) Longitudinal section of abnormal embryosac that stopped dividing at the fourth nuclear stage 2 days after pollination (Chikei 682-11×Kyushu No. 58) (×50). The bars indicate 100 μm (A, B, C, D).
ct; conducting tissue, edn; endosperm nucleus, em; embryo proper, ems; embryosac mp; micropyle, n; nucleus, ov; ovule, ova; ovary, pt; pollen tube, se; septum, su; suspensor

Table 5. Number of pollen tubes on stigma, upper style and ovary (Experiment 2)

Cross combination		Number of ovaries examined	Number of pollen tubes					Mean number of seeds per capsule
Female	Male		Stigma		Upper style		Ovary	
Kyushu No. 58 (C)	Chikei682-11 (A)	14	10.8	→ (37.0)[1)]	6.8	→ (7.4)	6.3	1.83
Chikei 682-11 (A)	Kyushu No. 58 (C)	14	13.6	→ (47.8)	7.1	→ (59.1)	2.9	1.76
Hayato (B)	Kyushu No. 58 (C)	12	11.0	→ (46.4)	5.9	→ (27.1)	4.3	1.42
Tsurunashigenji (B)	Kyushu No. 58 (C)	11	8.8	→ (53.4)	4.1	→ (36.6)	2.6	1.97
Minamiyutaka (B)	Kyushu No. 58 (C)	9	11.8	→ (48.3)	6.1	→ (60.7)	2.4	1.42
Koganesengan (B)	Kyushu No. 58 (C)	11	9.8	→ (49.0)	5.0	→ (44.0)	2.8	2.00
Norin No. 2 (B)	Kyushu No. 58 (C)	12	4.7	→ (23.4)	3.6	→ (8.3)	3.3	1.49
Benikomachi (E)	Kyushu No. 58 (C)	15	11.2	→ (48.2)	5.8	→ (41.4)	3.4	1.33
Kokei No. 14 (E)	Kyushu No. 58 (C)	14	11.6	→ (45.7)	6.2	→ (1.6)	6.1	1.54
FV 62-41 (J)	Kyushu No. 58 (C)	10	10.9	→ (23.9)	8.3	→ (11.2)	7.3	1.06

(A~J): Cross-incompatible group

[1)] Reduction rate in the number of pollen tubes %

Table 6. Number of pollen tubes penetrating the conducting tissue of ovary at 7 hours after pollination (Experiment 1)

Cross combination		Number of ovaries examined	Number of pollen tubes												Mean number of pollen tubes	Number of seeds per capsule	Percentage of capsules set
Female	Male		0	1	2	3	4	5	6	7	8	9	10	11			
Kyushu No. 58 (C)	Chikei682-11 (A)	16	1 (6.2)[1)]		2 1 (18.8)		2	3	3	3	1 (75.0)				4.8	2.04	79.8
Chikei682-11 (A)	Kyushu No. 58 (C)	14						2	3	2	3	1	1	2 (100.0)	7.6	2.11	84.6
Kyushu No. 58 (C)	F683-4 (B)	16			2 (12.5)		4	3	1	4	1 (87.5)	1			5.4	1.91	80.0
F683-4 (B)	Kyushu No. 58 (C)	31	12 (38.7)	3	7 (48.4)	5	2	1	1 (12.9)						1.7	1.00	10.3

(A~C): Cross-incompatible

[1)] Percentage

though there was relationship between the number of pollen tubes penetrating the conducting tissue and the number of seeds per capsule, there was no obvious connection between them. For example, in FV62-41 × Kyushu No. 58, the number of pollen tubes was the highest, 7.3 (Table 8), whereas the number of seeds was the lowest value, 1.06.

These results indicate that the reduction in the number of pollen tubes penetrating the conducting tissue is not a major cause of the low seed set, and does not show a close relationship with the number of seeds per capsule.

Relationship between embryosac with or without penetration of the pollen tube and embryo development 2 days after pollination

As indicated above, there were no major causes of low seed set in the process from pollen germination on the stigma to the penetration of pollen tubes into the conducting tissue of the ovary. Then, the author observed the relationship between the number of embryosacs into which the pollen tube had penetrated and the number of embryosacs in which the embryo normally developed after penetration of the pollen tube, 2 days after pollination. As stated in the previous paper, 2 days after pollination, the fertilized egg cells normally develop into both embryo proper and suspensor, and the fertilized polar nuclei develop normally into free endo-

Table 7. Number of pollen tubes penetrating the conducting tissue of ovary at 7 hours after pollination (Experiment 2)

Cross combination		Number of ovaries examined	Number of pollen tubes													Mean number of pollen tubes	Number of seeds per capsule	Percentage of capsules set
Female	Male		0	1	2	3	4	5	6	7	8	9	10	11	12			
Kyushu No. 58 (C)	Chikei682-11 (A)	14			1		2	2	4	1	2	1			1	6.3	1.76	75.9
					(7.1)[1)]						(92.9)							
Chikei682-11 (A)	Kyushu No. 58 (C)	14	1	1	3	5	2	2								2.9	1.83	60.0
			(7.1)		(64.3)			(28.6)										
Hayato (B)	Kyushu No. 58 (C)	12	1	1	1	1	1	3	2	2						4.3	1.42	57.0
			(8.3)		(25.0)				(66.7)									
Tsurunashi-genji (A)	Kyushu No. 58 (C)	11		3	1	4	3									2.6	1.97	61.2
					(72.7)		(27.3)											
Minamiyutaka (B)	Kyushu No. 58 (C)	9		2	2	4	1									2.4	1.42	49.2
					(88.9)		(11.1)											
Koganesengan (B)	Kyushu No. 58 (C)	11	1	1	3	2	2	2								2.8	2.00	76.4
			(9.1)		(54.5)			(36.4)										
Norin No. 2 (B)	Kyushu No. 58 (C)	12	1		3	5		1	1		1					3.3	1.49	52.3
			(8.3)		(66.7)				(25.0)									
Benikomachi (E)	Kyushu No. 58 (C)	15	1	3	1	2	4	1	2		1					3.4	1.33	48.8
			(6.7)		(40.0)				(53.3)									
Kokei No. 14 (E)	Kyushu No. 58 (C)	15		1	1	1	2	3	3	3		1				6.1	1.54	44.8
					(13.3)				(82.7)									
FV 62-41 (J)	Kyushu No. 58 (C)	10		1		1	1	2			1	1	1	1	1	7.3	1.06	58.7
					(10.0)						(90.0)							

(A~J): Cross-incompatible group

[1)] Percentage

sperm nuclei forming the peripheral layer of the embryosac (Fig. 1C) (Kokubu *et al.* 1982).

The percentage of embryosacs with or without penetration of the pollen tube is shown in Fig. 2. Although in Koganesengan × Kyushu No. 58, the percentage of embryosacs into which the pollen tubes had penetrated showed the highest value of 38.6% among the cross combinations, such a value was not particularly high.

Therefore, in order to determine the factors affecting the low seed set in each process as mentioned above, the rate of the change in the number of embryosacs penetrated by pollen tubes and the number of normally developed embryos was determined (Table 8). The number of proembryos was expressed using 2 kinds of parameters, [number of proembryos/number of flowers pollinated], and [number of proembryos / (number of flowers pollinated − number of ovaries in which proembryos did not grow)]. The number of seeds per capsule corresponded usually to the latter parameter, because the current investigation dealt with the ovaries, which set the capsules.

Among all the cross combinations, a very small number of embryosacs was penetrated by pollen tubes compared with the number of pollen tubes observed at 7 hours after pollination, and the rate of change in the reduction depended on the cross combinations.

As shown in Table 8, both the frequencies of the number of embryosacs penetrated by pollen tubes and the number of proembryos (number of proembryos/number of flowers pollinated) displayed very similar tendencies, suggesting that almost all the embryosacs penetrated by pollen tubes, formed proembryos. In most of the cross combinations, few pollen tubes penetrated the embryosac, because many pollen tubes stopped in the lower part of the ovary. It appears that the small number of embryosacs penetrated by pollen tubes accounted for the low seed set.

Furthermore, embryosacs without the penetration of a pollen tube were observed. Based on these observations, the embryosacs were almost all abnormal, in which cell division stopped at each stage of gametogenesis and the egg apparatus remained incomplete on the flowering day. The percentage of normal and abnormal embryosacs in the ovaries not penetrated by a pollen tube is shown in Fig. 3. In all the cross combinations, a high frequency of abnormal embryosacs was observed (Fig. 1D), indicating that the abnormality of the embryosacs was responsible for the lack of penetration of the pollen tubes on flowering days.

Relationship between the frequency of the number of seed sets per capsule and the number of embryos with normal development per ovary

To determine whether the proembryos 2 days after pollination developed into a complete embryo, the number of

Table 8. Number of embryosacs penetrated by pollen tubes and number of normally developed proembryos (Experiment 2)

Cross combination		Number of pollen tubes						Number of embryosacs penetrated by pollen tubes[3]	Number of proembryos	
Female	Male	Stigma[1]		Upper style[1]		Ovary[2]			for all the ovaries examined[4]	per ovary with developed proembryo[5]
Kyushu No. 58 (C)	Chikei682-11 (A)	10.8	→ (37.0)[6]	6.8	→ (7.4)	6.3	→ (76.2)	1.5	0.9	1.6
Chikei682-11 (A)	Kyushu No. 58 (C)	13.6	→ (47.8)	7.1	→ (59.1)	2.9	→ (55.2)	1.3	1.2	1.9
Hayato (B)	Kyushu No. 58 (C)	11.0	→ (46.4)	5.9	→ (27.1)	4.3	→ (83.7)	0.7	0.7	1.0
Tsurunashigenji (A)	Kyushu No. 58 (C)	8.8	→ (53.4)	4.1	→ (36.6)	2.6	→ (46.2)	1.4	1.3	1.6
Minamiyutaka (B)	Kyushu No. 58 (C)	11.8	→ (48.3)	6.1	→ (60.7)	2.4	→ (58.3)	1.0	0.8	1.2
Koganesengan (B)	Kyushu No. 58 (C)	9.8	→ (49.0)	5.0	→ (44.0)	2.8	→ (46.4)	1.5	1.5	2.3
Norin No. 2 (B)	Kyushu No. 58 (C)	4.7	→ (23.4)	3.6	→ (8.3)	3.3	→ (48.5)	1.7	1.3	1.7
Benikomachi (E)	Kyushu No. 58 (C)	11.2	→ (48.2)	5.8	→ (41.4)	3.4	→ (76.5)	0.8	0.7	1.1
Kokei No. 14 (E)	Kyushu No. 58 (C)	11.6	→ (45.7)	6.2	→ (1.6)	6.1	→ (85.2)	0.9	0.7	1.2
FV 62-41 (J)	Kyushu No. 58 (C)	10.9	→ (23.9)	8.3	→ (11.2)	7.3	→ (91.8)	0.6	0.5	1.0

(A~J): Cross-incompatible group

[1] Observed at 4 hours after pollination

[2] Observed at 7 hours after pollination

[3] Observed at 2 days after pollination

[4] Number of proembryos/number of flowers pollinated

[5] Number of proembryos/(number of flowers pollinated – number of ovaries in which proembryos did not grow)

[6] Reduction rate in the number of pollen tubes %

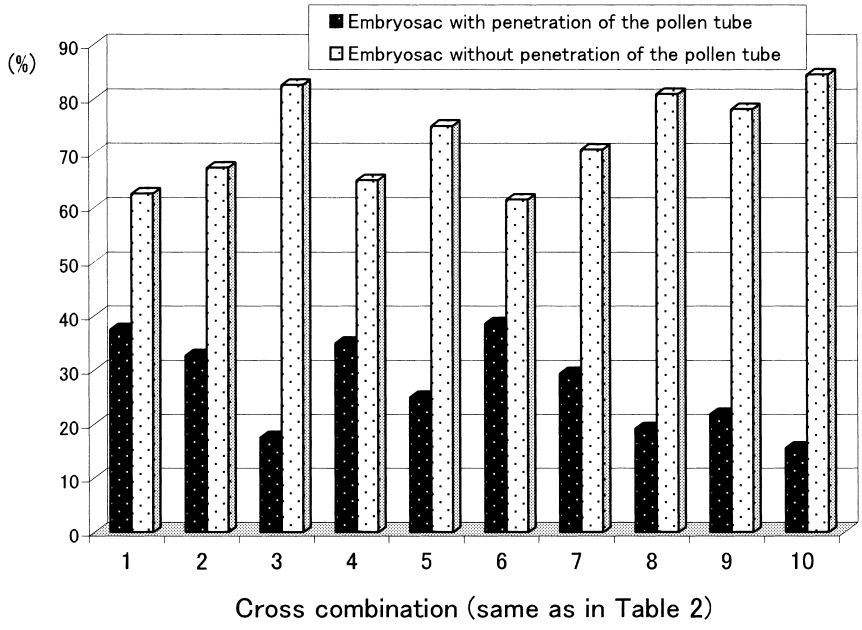

Fig. 2. Percentage of embryosacs with or without penetration of the pollen tube.

embryos showing a normal development 2 days after pollination per ovary compared with the number of seeds per capsule 30 days after pollination, was examined. The results are shown in Table 9. There was no significant difference between the number of proembryos per ovary and the number of seeds per capsule in all the cross combinations, suggest-

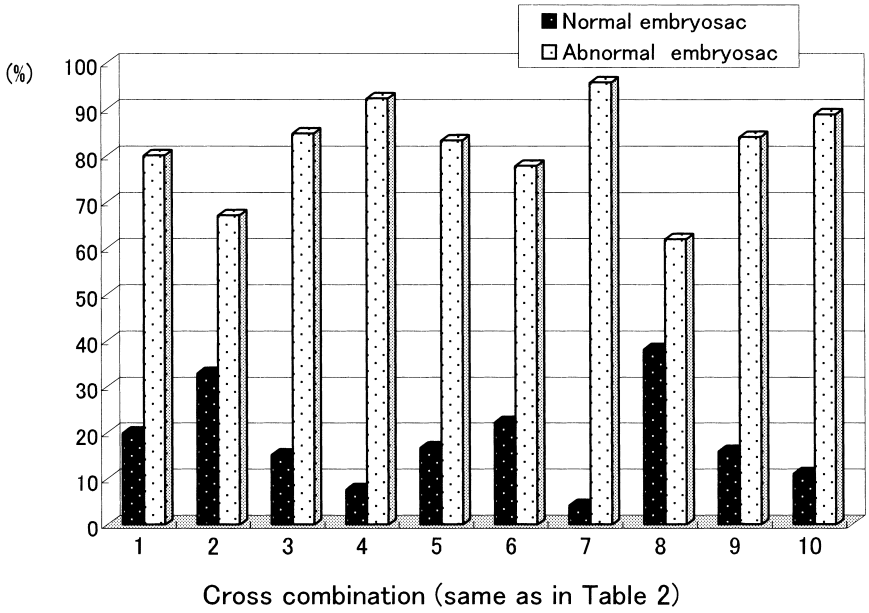

Fig. 3. Percentage of normal and abnormal embryosacs in the ovaries without penetration of the pollen tube.

Table 9. Relationship between the number of proembryos per ovary and the number of seeds per capsule (Experiment 2)

Cross combination		Number of proembryos per ovary				Mean number of proembyos (A)	Number of seeds per capsule				Mean number of seeds (B)	(A)-(B)
Female	Male	1	2	3	4		1	2	3	4		
Kyushu No. 58 (C)	Chikei682-11 (A)	5	0	2	0	1.57	38	28	14	2	1.76	–0.19
Chikei682-11 (A)	Kyushu No. 58 (C)	5	9	3	0	1.88	26	30	12	1	1.83	0.05
Hayato (B)	Kyushu No. 58 (C)	7	0	0	0	1.00	33	18	2	0	1.42	–0.42
Tsurunashigenji (A)	Kyushu No. 58 (C)	4	3	1	0	1.63	9	14	6	1	1.97	–0.34
Minamiyutaka (B)	Kyushu No. 58 (C)	4	1	0	0	1.20	20	9	2	0	1.42	–0.22
Koganesengan (B)	Kyushu No. 58 (C)	2	1	4	0	2.29	14	15	12	1	2.00	0.29
Norin No. 2 (B)	Kyushu No. 58 (C)	3	6	0	0	1.67	26	16	3	0	1.49	0.18
Benikomachi (E)	Kyushu No. 58 (C)	7	1	0	0	1.13	15	5	1	0	1.33	0.20
Kokei No. 14 (E)	Kyushu No. 58 (C)	7	2	0	0	1.22	7	5	1	0	1.54	–0.32
FV 62-41 (J)	Kyushu No. 58 (C)	4	0	0	0	1.00	16	1	0	0	1.05	–0.05

(A~J): Cross-incompatible group

ing that almost all the proembryos showing normal development 2 days after pollination, continued to grow until they reached maturity.

Discussion

Ting and Kehr (1953), and Wang (1964) reported that the low seed set in some lines of sweet potato may result from meiotic abnormalities and the increasing number of sterile pollen grains. However, Jones (1965) noted that since the cytological observations of 40 clones revealed that the meiotic activity of sweet potato was normal, the low seed set must be due to causes other than meiotic abnormality. Fujise (1963) reported that it was not conceivable that low seed sets were limited by few pollen grains. Futhermore, Martin and Ortiz (1965) pointed out that the low seed sets must be due to causes other than the number of pollen grains that germinated on the stigma. As stated above in this study, pollen fertility on flowering days exceeded 80% in most of the varieties examined and pollen germination 4 hours after pollination was in the range of 10~20% in most of the crosses. These results indicate that neither the fertility of mature pollen nor pollen germination on the stigma is the principal cause of the low seed set.

Martin and Cabanillas (1966) reported that of the 3 sweet potato cross combinations selected, adequate pollen germination was characterized by a moderate to a large difficulty in the passage of the tubes from the stigma to the style. About 6 times as many tubes penetrated the style compared with the number of seeds produced. Furthermore, Martin and Ortiz (1967) noted that sterility might be due to pollen tube disorientation or failure at the stigma-style junc-

tion and the style-ovary junction, where passage may be mechanically more difficult. As shown in Table 6 and Table 7, the mean number of pollen tubes penetrating the conducting tissue of the ovary 7 hours after pollination differed considerably among the cross combinations. However, no close relationship with the number of seeds per capsule was revealed.

Among all the cross combinations, a very small number of pollen tubes penetrated the embryosac compared with the number of pollen tubes that penetrated the conducting tissue. Embryosacs not penetrated by a pollen tube were almost all abnormal, in which the egg apparatus had been stopped halfway through gametogenesis. In previous experiments, histological observations were conducted on the normal or abnormal processes of gametogenesis of sweet potato (Murata 1986). Higashiyama *et al.* (1998) and Higashiyama *et al.* (2001) reported that the synergid cells of the female gametophyte played an important physiological function in *Torenia fournieri*. When two synergid cells were ablated by a UV laser beam, they observed that no embryosac attracted a pollen tube and at least one synergid cell was necessary for pollen tube attraction.

On the other hand, both the frequency of the number of proembryos observed 2 days after pollination and the number of seeds per capsule, showed a very similar tendency. These results indicate that almost all the proembryos continued to grow until they reached maturity.

These histological observations showed that the principal cause of the low seed sets of sweet potato was the large number of such abnormal embryosacs.

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