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The Behaviour of Pollen Tubes on the Stigma in the Intersectional Crosses in *Populus* and Its Relation to Seed-setting *

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Abstract

The behaviour of pollen tubes on the stigma between intersectional crosses in *Populus* has been examined with light fluorescence microscope and scanning electron microscope. The results are as follows : both normal and abnormal pollen tubes are present simultaneously in all intersectional combinations ; usually, the more abnormal tubes and the heavier callose deposits appear, the more difficult the cross is ; some of the combinations which have a great number of abnormal tubes and thick callose deposits may produce seeds, while others with many short tubes and less callose deposits set no seed at all. It is evident that the quantity of abnormal pollen tubes and callose deposit response may reveal the cross-difficulty in some intersectional crosses, but can not be used as a diagnostic indicator for rapid assessment of seed production .

Key words : *Populus*, Cross, Pollen tube, Stigma

Introduction

Pollen-stigma interaction is the first step of the sexual process. It's easy to think that the performance of this interaction may be a useful signal for compatibility and seed setting. In the past two decades, there were a number of studies in this field. With regard to *Populus*, Knox and his co-workers reported : a recognition protein released by the pollen wall set the stage for germination and tube growth (Knox et al. , 1972a); the twisted and extended pollen tubes and heavy callose deposits showed an incompatible matings (Knox et al., 1972b); the callose response provided us a diagnostic indicator for rapid assessment of the nature of a pollination in the biotechnology of seed production (Dumas & Knox, 1983). At variance with their reports, Stettler and his co-workers (1980) described: in six of seven intersectional cross combinations observed, pollen tubes behaved on foreign stigmas essentially the same way as in conspecific matings, except for that the percentage of twisted and bulging tubes was significantly higher. Later, Li and Zhu (1986) found that *P. simonii*

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× *P. euphratica*, a well-known cross-difficult combination, set a few hybrid seeds, in spite of the presence of a large numbers of twisted tubes and heavy callose deposits; while *P. euphratica* × *P. simonii*, an incompatible combination showing a lot of short tubes and less twisted tubes sets no seed at all. These phenomena encouraged us to make a thorough examination of the behaviour of pollen tubes on stigma in the intersectional crosses of *Populus* in order to explore its influence on seed-setting.

Materials and Methods

Parent plants and artificial pollination Male and female branches of poplar species were collected from various parts of China as listed in Table 1

Table 1. Parent plants used in this experiment

Section	Species	Locality
Tacamahaca Spach	*Populus simonii* Carr.	Beijing, Shanxi
Aigeiros Duby	*P. nigra* L.	Shanxi
Leuce Duby	*P. alba* L.	Beijing
	P. adenopoda Maxim.	Nanjing
Leucoides Spach	*P. lasiocarpa* Oliv.	Hubei
Turanga Bge	*P. euphratica* Oliv.	Inner Mongolia Ningxia

All the detached male and female branches were in cellar-storage. To ensure the purity of pollen grains, male branches of various species were cultured in water in isolated rooms. Pollen was stored in refrigerator at the temperature 2 — 4°C. The buds of female inflorescence were covered with transparent paper before anthesis to avoid contamination. Artificial pollination was conducted at 10 :00 — 11 :00 a.m. or 1 :30 — 2 :30 p.m.

P. simonii, *P. nigra*, *P. alba*, *P. lasiocarpa* and *P. euphratica* were used as female plants. Each was dusted with pollen from the species of the other four sections. All were water-cultured until the fruits were ripened (compatible crosses) or the pollinated catkin was come off (incompatible crosses).

Samples preparation

Female flowers were fixed in FAA or 2.5% GA at 4, 8 and 12 h after pollination. For light fluorescence microscopy, squash preparations (Martin, 1959) and serial paraffin sections were stained with 0.01 — 0.05% decolorized aniline blue. Samples were observed under AO 2071 incident light fluorescence microscope, 2073 flour cluster (Exciter 436 mm, Dichooic 450 mm, Barrier GG 475). For SEM, the fixed female flowers or stigmas were dehydrated in alcoholic series prior to critical point drying with CO_2, mounted on stubs and sputted-coated with carbon and gold. Samples were observed in Philips S505 SEM.

Observations and Results

1. Stigmas and germinated pollen grains

The female poplar flower consists of one pistil with a cup-shaped perianth (Hong et al., 1987) and subtend in a bract. Most styles are short, except that those of *P. lasiocarpa* are rather long. Stigmas are usually 2 — 5; *P. simonii* and *P. nigra* 2-lobed, horseshoe-shaped; *P. alba* 2-parted, linear-forked; *P. euphratica* and *P. lasiocarpa* 3-5-bladed, petaloid and crackled. The stigmatic surface cells are dome-shaped, often aggregate to form multicellular papilla, secreting at the receptive stage (P1. I : 1 — 3).

In order to prove the purity of the dusted pollen grains, the morphology of the germinated pollen grains of 6 *Populus* species is observed and recorded (P1. I : 4 — 9).

2. The behaviour of pollen tubes, callose deposits and seed-setting. An overview of the results are given in Table 2. Terms used are according to Stettler et al. (1980) and Williams et al. (1982).

(1) Conspecific matings Soon after landing on the stigma, most of the pollen grains germinate and the tubes penetrate into the stigma through the intercellular space or occasionally enter its outer cell wall directly. Most of the tubes are short, only a few of them are long, extended and twisted, and may enter the stigma ultimately. No extremely twisted, coiled, winded, swollen or bursted tubes have been found. A series of callose plugs are formed at a regular distance behind the tube tip, but terminal, heavy and full callose plugs have never been seen. The presence of a few extended and twisted tubes does not affect seed-setting. All of the 5 combinations of conspecific matings set well (P1. I : 10 — 12 ; P1. III : 25).

(2) Intersectional crosses In addition to short tubes, there are various kinds of abnormal tubes such as extended, twisted, winded, coiled, swollen bursted tubes, etc. Of all the 20 crosses, twisted and short tubes exist simultaneously. In the incompatible combinaations, stigma callose response rarely occurs (P1. II : 13 — 24; P1. III : 26 — 36).

The ratio of short tubes to abnormal ones is different in various combinations. Generally the more abnormal tubes and heavier callose deposits appear, the more difficult the cross is , as : *P. simonii* is pollinated respectively with pollen of the other 4 sections, an order of compatibility may be arranged as follows according to the behaviour of pollen tubes, the deposition of callose and the quantity and quality of the hybrid seeds (P1. II : 13 — 15 ; P1. III : 26 — 30).

In crosses where *P. euphratica* is used as male parent, the pollen tubes are extremely abnormal on foreign stigmas : wandering over the stigmatic cells for a long distance; twisting to give a spiral form ; coiling round and round; bulging to form an irregular swelling or curve appearence. Terminal, heavy and full callose plugs often deposit in the extremely abnormal tubes (P1. II : 18, 21; P1. III : 28 — 30, 33). In three of four attempted crossings, no seed has been set at all. There is one exception, *P. simonii* × *P. euphratica*. which has a few hybrid seeds and their seedlings growing very weak.

(3) Although the quantity and behaviour of abnormal tubes may reveal cross-difficulty, yet the presence of abnormal tubes can not be considered as a signal for incompatibility.

Table 2. Behaviour of pollen tubes, callose deposits and seed-setting

Crosses	No. of photos	Total no. of tubes	Tubes behaviour														Callose deposits				Seed-setting
			Short		Long		Extended		Twisted		Winded		Swollen		Bursted		Normal	Terminal	Heavy	Full	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%					
P. simonii × *P. simonii*	3	70	69	98.57	1	1.43											+				✓
P. simonii × *P. lasiocarpa*	6	133	117	87.96	1	0.75	1		8	6.0			4	3.0	2	1.5	+				✓
P. simonii × *P. nigra*	6	99	80	80.80			9	9.09	10	10.10							+	+			✓
P. simonii × *P. adenopoda*	5	43	27	62.79			1	2.33	11	25.58	3	6.98			1	2.33	+		+		✓
P. simonii × *P. euphratica*	11	87	17	19.54			14	16.09	48	55.17			2	2.3	6	6.9	+	+	+	+	✓
P. nigra × *P. nigra*	2	74	68	91.89			6	8.11									+				✓
P. nigra × *P. lasiocarpa*	5	78	62	79.49			6	7.69	10	12.82							+				✓
P. nigra × *P. simonii*	3	71	54	76.06	8	11.27	3	4.23	3	4.23			2	2.82	1	1.41	+			+	✓
P. nigra × *P. adenopoda*	3	69	49	71.01			6	8.70	14	20.29							+				✓
P. nigra × *P. euphratica*	2	71	27	38.03					41	57.75			3	4.23			+	+	+	+	0
P. alba × *P. alba*	2	12	12	100													+				✓
P. alba × *P. nigra*	1	7	4	57.14			1	14.29	2	28.57							+				0
P. alba × *P. lasiocarpa*	2	26	12	46.15			1	3.85	13	50							+		+		0
P. alba × *P. simonii*	9	89	37	41.57			24	26.97	26	29.31			1	1.12	1	1.12	+	+	+		✓
P. alba × *P. euphratica*	7	27	11	40.74			8	29.63	8	29.63							+	+			0
P. lasiocarpa × *P. lasiocarpa*	3	54	52	96.3					2	3.70							+				✓
P. lasiocarpa × *P. nigra*	6	35	30	85.71			1	2.86	4	11.43							+				0
P. lasiocarpa × *P. simonii*	8	31	29	93.55	1	3.23	1	3.23	4	12.90							+	+			0
P. lasiocarpa × *P. adenopoda*	7	33	26	78.79					4	12.12					3	9.09	+				0
P. lasiocarpa × *P. euphratica*	7	68	21	30.88			25	36.76	19	27.94			3	4.41			+	+			0
P. euphratica × *P. euphratica*	4	71	70	98.59	1	1.41											+				v
P. euphratica × *P. nigra*	6	30	27	90.0			1	3.33	1	3.33					1	3.33	+	+	+	+	0
P. euphratica × *P. alba*	5	13	11	84.62					2	15.38							+	+			0
P. euphratica × *P. simonii*	11	78	59	75.64	4	5.13			3	3.85			1	1.28	11	14.10	+				0
P. euphratica × *P. lasiocarpa*	6	27	19	70.37	2	7.41			2	7.41			4	14.81			+				0

Notes : Based on SEM photos, all in the same magnification, ×422. + : present, ✓ : seed-setting, 0 : no seed-setting.

Among the twenty cross combinations, there are eight ones which produce hybrid seeds.

Table 3. Compatibility order between *Populus simonii* and species of the other 4 sections

Compatibility order	*P.simonii* × *P.lasiocarpa*	>	*P.simonii* × *P.nigra*	>	*P.simonii* × *P.adenopoda*	>	*P.simonii* × *P.euphratica*
*Short tubes/ abnormal tubes	87.75/ 13.05		80.80/ 19.19		62.79/ 37.22		19.54/ 78.46
Abnormality of tubes	Slight		Moderate		Serious		Extreme
Callose deposits	Normal		Normal		Occassionally heavy		All abnormal kinds present
Seed	Most well-developed with full embryo		Most well-developed with full embryo		Well or poor-developed, embryos full or not		A large number of abortive seeds with embryos not full
Sedling	Strong		Strong		Weak		Very weak

The number of abnormal tubes are more than that of short tubes : *P.simonii* × *P.euphratica* and *P.alba* × *P.simonii* ; The number of abnormal tubes are approximately equal to that of short tubes, such as *P.simonii* × *P. adenopoda* ; The number of abnormal tubes are less than that of short tubes : *P.simonii* × *P.nigra*, *P.nigra* × *P.lasiocarpa* etc.

(4) Usually, a large number of short tubes reveal cross-compatibility in some combinations, like using *P. simonii* (Sect. Tacamahaca) and *P. nigra* (Sect. Aigeiros) as female parents. In contrast, in the crosses with *P. lasiocarpa* and *P. euphratica* as female plants, no seed has been set at all in spite of the fact that there are more short tubes than the abnormal tubes and the callose deposits are not heavy (P1. II :22 — 24 ; P1. III :34 — 35).

Discussion

A review of characteristics of the rejection reaction in self-incompatibility system by Heslop-Harrison (1975) read: the pollen tube grows in an abnormal manner and is occluded by callose; the stigma papillae deposit callose rapidly, which seems to be an exclusive feature of sporophytic self-incompatibility systems. The reaction is so consistent that it can be used as a bioassay for incompatibility.

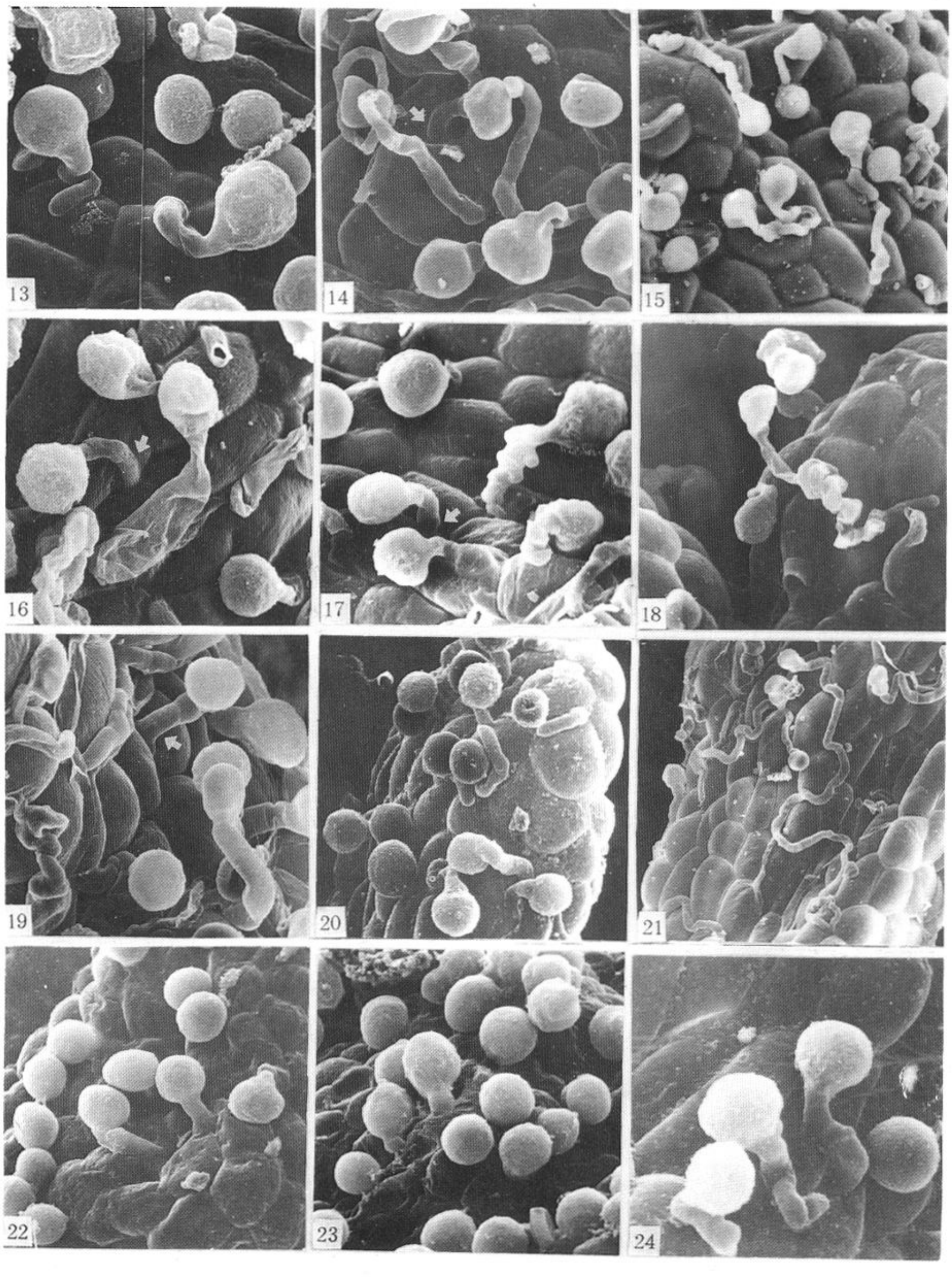
Can the twisted tubes and callose deposits be used as an index of bioassays for the intersectional incompatibility in *Populus* ? In their studies on the intersectional incompatible matings in *Populus*, Knox and his colleagues (1972b) reported that in *P. deltoides* × *P. alba*, callose is localized in the pollen tubes which had grown over the surface for varying distances and had not penetrated, while in *P. tremuloides* × *P.euramericana*, only a few grains had produced short coiled tubes that failed to penetrate the stigmatic surface (Gaget et al., 1984). In short, they considered that the expression of incompatible intersectional pollinations in poplars is the extended or coiled pollen tubes on the stigmatic surface with heavy callose deposits.

After reviewing the callose response in interspecific matings, Dumas and Knox (1983) concluded that the phenotype of the response is similar to that of the self-incompatibility. And, they considered that the callose response provides a useful indicator for the biotechnology of seed production. A subsequent report (Gaget et al., 1984) declared; "In the section Leuce, the phenotype of incompatibility responses is typical of a sporophytically controlled system occurring at the stigma surface. On the contrary, in other sections, Aigeiros and Tacamahaca, the responses are typical of a gametophytically controlled system, occurring mainly in the style."

The results of our experiment are not in harmony with the earlier workers'. Obviously in some of the intersectional crosses of *Populus*, the more abnormal tubes and the heavier callose deposits appear, the more difficult the cross is; whereas in some other combinations, in spite of the presence of abnormal tubes, the hybrid seed setting is good. And why? There are two prominent features in all of the twenty intersectional crosses in this experiment, i.e. (1) the simultaneity of short tubes and twisted tubes; (2) some of the twisted tubes and the extended tubes may ultimately penetrate into the stigma. These phenomena suggest the possibility of entrance of pollen tubes into the ovarian cavity and the occurrence of fertilization. It is true that in the sexual process of the intersectional crosses we observed, such as in *P. simonii* × *P. euphratica* and *P. alba* × *P. simonii*, fertilization did take place and hybrid seeds resulted even though there were a large number of abnormal tubes and heavy callose deposits. It follows that the appearance of twisted tubes and callose deposits in the intersectional crosses in *Populus* cannot be a signal for incompatibility due to the simultaneity of the short tubes. It is therefore questionable to affirm that it is incompatible when twisted tubes and callose deposits are observed, and that it is compatible when a great number of short tubes are present. There is still a long way to go to reach seed-setting after the pollen lands on a foreign receptive stigma surface. For example, in the combination of *P. euphratica* × *P. simonii*, pollen tubes entered the ovarian cavity but no fertilization occurred (Li & Zhu, 1986) while in the combination of *P. alba* × *P. lasiocarpa* or *P. alba* × *P. euphratica* fertilization occurred but hybrid embryos aborted at various developmental stages. It is evident that a series of barriers may appear in the subsequent courses after the pollen tubes enter the ovary. Therefore, it is unsatisfactory to pay attention only to the quantity of short tubes but neglect the later events incompatibility (Seavey & Bawa, 1986).

With regard to the pollen-stigma callose response, we have found that there were a few callose dots occasionally appearing in some combinations. It is doubtful that the callose dots were mechanical wounds. Because they were not in contact with the adjoining pollen. It is believed that there are no stigma callose response in the incompatible intersectional crosses in *Populus*.

We also doubt Gaget's explanation (1984) because the mechanisms of self-incompatibility and distant hybridization are quite different. And our result showed that in the crosses of section Leuce and other four sections, the pollen tubes could more or less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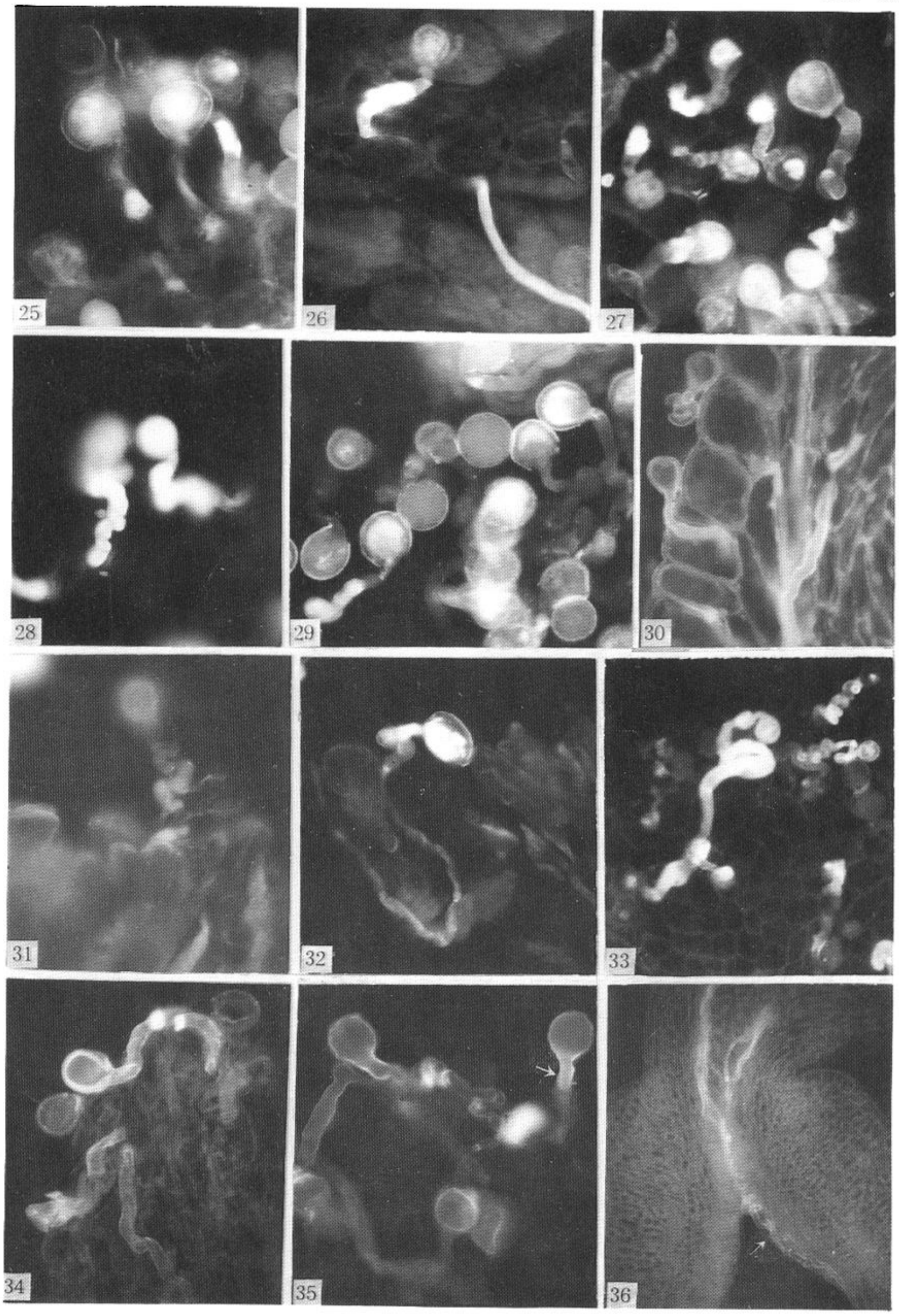
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